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Volume 84, No. 995 February 1959

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PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, February 4th, 1959, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. J. H. Hamence, M.Sc., F.R.I.C.

The subject of the meeting was "The Estimation of Pesticide Residues" and the following papers were presented and discussed: Introductory Talk by G. L. Baldit, B.Sc., A.R.I.C.; "The Determination of Residues of Systemic Organo-phosphorus Insecticides in Vegetables," by E. Q. Laws, B.Sc., F.R.I.C.; "The Determination of Residues of Aldrin, Dieldrin and Endrin," by J. G. Reynolds, F.R.I.C.; "The Determination of Organo-mercury Residues in Plant Material," by M. G. Ashley, F.P.S., F.R.I.C.

NEW MEMBERS

Ordinary Members

Frederick James Bryant, B.Sc., Ph.D. (Lond.), A.R.C.S., D.I.C.; Freshfield Leonard Cann, B.Sc. (Lond.), A.R.I.C.; Geoffrey Clewlow, B.Sc. (Manc.); John Clifford, B.Sc., M.A. (Oxon.); Joyce Cuckney; Michael Deasy, A.R.I.C.; Alexander Dym, B.Sc. (Lond.); John Louis Hague, B.S. (Washington); John Hawthorn, B.Sc., Ph.D., A.R.C.S.T., F.R.I.C.; Anthony Robert Jeffs; Henry Alexander Kahn; Henry Leach, F.I.M.L.T.; Melville Henry Litchfield, B.Sc. (Lond.), A.R.I.C.; Maarten J. Maurice, Dr. Chem. (Amsterdam); Joseph Henry Ambrose Ruzicka, B.Sc. (Lond.), A.R.I.C.; Harold Douglas Thornton, B.Sc. (N.U.I.), A.R.C.Sc.I., F.I.C.I., F.R.I.C.; Thomas Charles Gill Whitehead, B.Sc. (Lond.), A.R.I.C.; Arthur John Woodgate, B.Sc. (Lond.); Rex Beville Woo-Ming, B.Sc. (Lond.).

Junior Members

Alan Walker, A.M.Inst.S.P., A.R.S.H.; Thomas George Whiston.

DEATHS

We record with regret the deaths of

Herbert John Evans

Ernest Wilfrid Jackson.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 6.30 p.m. on Tuesday, January 13th, 1959, in the Mason Theatre, The University, Edmund Street, Birmigham, 3. The Chair was taken by the Chairman of the Section, Dr. R. Belcher, F.R.I.C., F.Inst.F.

A discussion on "The Analytical Chemistry of Cobalt and Nickel" was opened by A. J. Brookes, A.R.I.C., A.C.T.

SUMMARIES OF PAPERS PRESENTED AT A MEETING OF THE SCOTTISH SECTION

THE following are summaries of two papers presented at the Ordinary Meeting of the Scottish Section on Wednesday, November 19th, 1958, in Glasgow. A first report appeared in *The Analyst*, 1958, **83**, 654.

The subject of the meeting was "Developments in Gas Chromatography," and the papers were: "Quantitative Analysis Using Thermal Conductivity Detection," by G. R. Jamieson, B.Sc., F.R.I.C.; "The Application of Gas Chromatography to Gas Reaction Kinetics," by J. H. Knox, B.Sc., Ph.D.; "Chromatographic Examination of a Low-temperature Tar," by L. Irvine, B.Sc., Ph.D., A.R.C.S.T., A.R.I.C. (The work described in the third paper has already been published by L. Irvine and T. J. Mitchell, *J. Appl. Chem.*, 1958, 8, 425).

QUANTITATIVE ANALYSIS USING THERMAL CONDUCTIVITY DETECTION

MR. G. R. JAMIESON said that for quantitative analysis when nitrogen was used as the carrier gas the detector had to be calibrated for each component of the mixture, since there was not, as a rule, a linear relationship between the response of a katharometer and the amount of a component in the carrier gas. Both positive and negative deviations from linearity could occur (A.I.M. Keulemans, A. Kwantes and G. W. A. Rijnders, *Anal. Chim. Acta*, 1957, **16**, 29). It was advantageous to use a carrier gas that had a thermal conductivity greatly different from any of the components to be determined. With helium or hydrogen as the carrier gas the non-linearities were much smaller, but even with these gases calibration factors had to be used for the highest accuracy.

There seemed to be less need for calibration as the operating temperature was increased; *e.g.*, the methyl esters of fatty acids from C_9 to C_{22} had been analysed satisfactorily without calibration with nitrogen as the carrier gas.

In quantitative analysis making use of peak areas it was important to take into account the "surge effect," although this effect was very difficult to calculate (F. van de Craats, Preprints of Second Symposium on Gas Chromatography, p. 123).

A correction for the thermal conductivity of the component had been proposed (L. C. Browning and J. O. Watts, *Anal. Chem.*, 1957, **29**, 24), but this had not been found useful with nitrogen as the carrier gas. A drawback to the use of this correction was that the thermal conductivities of most organic compounds were not recorded for different temperatures.

With helium as the carrier gas, a direct relationship had been found between detector response and the square root of the molecular weight (R. H. Eastman, *J. Amer. Chem. Soc.*, 1957, **79**, 4243), but with nitrogen as the carrier gas this relationship had only been found to hold for members of an homologous series, *e.g.*, *n*-alkyl acetates from methyl acetate to *n*-amyl acetate, and for benzene, toluene and the xylenes. In an interesting paper (D. M. Rosie and R. L. Grob, *Anal. Chem.*, 1957, **29**, 1263) it was shown that compounds with similar compositions and structures gave similar detector responses, *e.g.*, benzene, 100; *o*-xylene, 130; *m*-xylene, 131; *p*-xylene, 131; 2 : 2-dimethylbutane, 116; 2 : 3-dimethylbutane, 116.

A method that was being used more frequently was to pass the components, after separation on the column, through a small combustion furnace containing copper oxide. The products of combustion, carbon dioxide and water, then passed to the detector. The advantages of this method were—

(*a*) the effect of any differences in thermal conductivity between different components was overcome;

(*b*) there was no contamination of detectors or of vent lines;

(*c*) a particular advantage for the detection of minor components was the increase in sensitivity. Sensitivity was also aided by the fact that the detector could be operated at low temperatures—just high enough to keep the products of combustion in the vapour state; and

(*d*) satisfactory results were obtained with easily constructed detectors (W. Stuve, Preprints of Second Symposium on Gas Chromatography, p. 1).

A novel use of the combustion method was to have the furnace before the column and to separate the products of combustion on the column. The recorded chromatogram was then used to determine the carbon - hydrogen ratio.

A special reactor - gas chromatography unit had been reported for the analysis of amino acids (A. Zlatkis and J. F. Oró, *Anal. Chem.*, 1958, **30**, 1156). With hydrogen as the carrier gas, the amino acids were injected into a column of ninhydrin on Celite, where they were oxidised immediately to aldehydes, which were then separated on a silicone - Celite column. As each aldehyde left the column it was cracked over a nickel - kieselguhr catalyst at 425° C to methane and water. After removal of the water in a small Drierite column, the methane passed to the detector. Calibration was unnecessary. Glycine could not be used in this method since the formaldehyde formed polymerised on the column.

The Application of Gas Chromatography to Gas Reaction Kinetics

Dr. J. H. Knox said that the many difficulties encountered in applying standard analytical techniques to experiments in gas reaction kinetics had been largely overcome by the development of gas chromatography, and Dr. Trotman-Dickenson and he were using this technique in Edinburgh for elucidating a wide variety of problems in gas kinetics. These included hydrocarbon oxidation, the reactions of methylene, the reactions of alkyl and alkoxy radicles, the rates of decomposition and isomerisation of organic compounds and the competitive fluorination, chlorination and bromination of hydrocarbons. Many of these problems could not have been tackled without the aid of gas chromatography. Its advantages over the standard methods were many, some of the more important being—

(i) the amounts of sample normally required were extremely small: 10^{-4} to 10^{-6} mole with conventional detectors and 10^{-9} mole with the ionisation type of detector;

(ii) the identity of the components of any mixture did not need to be known with certainty before the analysis was made. The pure components could be identified after they had been separated on the column; and

(iii) the proportional accuracy with which any component could be determined was independent of the absolute amount present.

When used for work in gas kinetics, certain modifications of the standard apparatus were often necessary. Generally, the contents of a reaction vessel or sampling vessel had to be transferred completely to the column. This could not be done by the usual syringe - serum cap method of injection; instead, a by-pass U-tube was used, into which the sample was transferred quantitatively. The sample could then be injected on to the column by switching the U-tube into the main carrier-gas stream.

The nature of the products from kinetic experiments presented two other problems of general importance. It was often desirable to carry out reactions to such a small percentage conversion that the vital products must be determined in the presence of a vast excess of unreacted starting material. It was often convenient to remove the unwanted reactants by means of a fore-column containing a suitable adsorbent, and solutions of inorganic salts in glycerol, supported on firebrick, had proved suitable. Another problem, the analysis of very volatile components, such as ethane and methane, along with liquids, such as isomeric hexanes, could not be carried out on a single column, but required a two-stage column consisting of a firebrick partition column followed by an adsorption column. Timing was important in the use of multistage columns, but suitable variations of the technique to solve particular problems could readily be devised.

An important point in the application of gas chromatography to analysing very small samples containing unknown components was the identification of the peaks recorded on the chromatogram. For amounts of the order of 10^{-6} to 10^{-7} mole there appeared to be only two methods of general applicability: infra-red spectroscopy and mass spectrometry. The former had recently been applied successfully by Dr. D. M. W. Anderson, at Edinburgh, to the identification of amounts in this range, and the development of these identification techniques would become of increasing importance as more sensitive gas-chromatographic units became widely used.

Obituary

ALEXANDER HUTCHESON BENNETT

ALEXANDER HUTCHESON BENNETT died on January 25th, 1958. He was born in Edinburgh in 1874 and, after taking his degree in chemistry, started his career in the laboratories of John Bennet Lawes & Company, Millwall, Manufacturers of Citric and Tartaric Acids.

This firm was later taken over by Kemball Bishop & Co., Ltd., for whom Bennett acted as consultant up to the time of his death.

In 1900 Bennett became a partner of Ogston & Moore, Analytical Consulting Chemists, who specialised in citrus products. In 1905 he took charge of the laboratories of Ogston & Moore in Messina, and he spent the greater part of his life in Sicily until the outbreak of the second World War.

Bennett was acknowledged as a high authority on the chemistry of citrus fruits and he acted in an advisory capacity to most of the Sicilian lemon growers.

Bennett contributed many papers to *The Analyst*. As far back as 1916 he published a paper entitled "The Estimation of Potassium in presence of other Substances." In 1922 he published a note dealing with the adulteration of liquorice paste and was the first chemist to describe the characteristics of a root known as Carlina Gummifera, the extract of which was then being admixed with liquorice paste.

In 1934 Bennett wrote a paper setting out the conditions necessary for estimating the vitamin C in citrus juices, and in 1942, in conjunction with F. K. Donovan, he wrote a further paper dealing with the determination of sulphur dioxide in citrus juices.

The writer had the pleasure of enjoying the friendship of Mr. Bennett for a number of years and always appreciated his kindly disposition and strong character.

Bennett was a lifelong member of the Chemical Society and the Society of Chemical Industry, and he joined what was then known as the Society of Public Analysts as far back as 1903. He never married; he was devoted to his sister, who predeceased him by about two years.

C. W. McHUGO

The Analysis of Synthetic Detergents

A Review*

By W. B. SMITH

(*Marchon Products Limited, Whitehaven, Cumberland*)

SUMMARY OF CONTENTS

DURING the past two decades, a wide range of synthetic materials has appeared on the market to displace soap from the unique position as a detergent that it has held for thousands of years. The introduction of new types of detergent is continuing, and with the subject still in a state of flux it is difficult to present a balanced review of its analytical aspects. This review, therefore, is unbalanced in the sense that only a brief treatment is given of the fields in which rapid developments, commercial or analytical, are proceeding (*e.g.*, ampholytic and certain non-ionic surface-active agents).

In order to increase the usefulness of the review, a critical approach has been adopted, and this may have introduced some further bias towards emphasis on subjects with which I am most familiar. Further, it should be pointed out that attention is centred on surface-active agents (surfactants) with a certain hydrophilic - hydrophobic balance that makes them useful as detergents. Surfactants with large hydrophobic groups, including surfactants that are insoluble in water, are considered as emulsifiers rather than detergents, and most of those with more than one hydrophilic group in the molecule are typical wetting-agents and are likewise dismissed from further consideration. By thus confining the review to detergents, narrowly defined as such, certain aspects can be dealt with in more detail than would otherwise be practicable.

No attempt at a complete coverage of the literature is made, the reader being referred to a recent bibliography[1] of the subject, although it is to be regretted that this is so poorly indexed. Other recent reviews[2,3,4] are, however, appropriately mentioned in this introductory section.

The American Society for Testing Materials has defined a detergent as "a composition that cleans," a basis that is too wide for this review, and the much narrower subject of the domestic spray-dried powder has been adopted as a central theme. The compounds that may be present in such a detergent are—

Surface-active constituent.
Non-ionic foam promoter.
Condensed phosphate.
Sodium silicate.
Sodium sulphate.
Sodium chloride.
Sodium carbonate.
Sodium perborate.
Ethylenediaminetetra-acetic acid.
Magnesium sulphate.
Carboxymethylcellulose.
Fluorescent dye, pigment and perfume.
Moisture.

* Based on a lecture delivered at the meeting of the Midlands Section on Thursday, March 13th, 1958. Reprints of this paper will be available shortly. For details, please see p. 122.

Magnesium sulphate or ethylenediaminetetra-acetic acid is generally added to stabilise the bleaching agent, sodium perborate. If perborate is absent, some amine or thioamide may be added as a tarnish inhibitor.

Although the spray-dried powder takes the greater share of the domestic market, many liquid detergents have been produced, and, if the subject is widened further to include the special detergents, toothpastes and shampoos, and also industrial cleaning agents, the following additional compounds must be considered—

Abrasive.
Glycerol.
Toluene or xylene sulphonate.
Urea.
Ethanol or *iso*propyl alcohol.

The last three ingredients are incorporated in liquid detergents to maintain clarity of the solution at low storage temperatures.

The surface-active constituent of most spray-dried powders is sodium dodecylbenzene sulphonate, although salts of primary and secondary alkyl sulphates are sometimes used. In liquids, on the other hand, the range of materials that may be present is very wide, and, besides the aforementioned sulphonate and sulphates, includes ethylene oxide derivatives of alcohols, alkylphenols and amides in both non-ionic and sulphated forms. In powders, only sodium salts are usually found, whereas in liquids, potassium, ammonium and mono- and triethanolamine salts are often encountered.

Qualitative tests for the inorganic compounds are briefly indicated in a later section; for fuller details, reference must be made to the original papers. Alternatively, simple adaptations of the methods of quantitative analysis that are dealt with in the final section of this review can be used. For the surface-active constituents, on the other hand, it is much more important to have qualitative information about their identity before quantitative analysis is embarked upon, and this is dealt with below.

Classification of surfactants

In the qualitative analysis of surfactants, it is essential that the investigator should have some idea of the types of compound that may be present, and the listing and classification of types is a pre-requisite for the construction of any analytical scheme. Classification can be based on structural considerations or on responses to a series of qualitative tests (compare the periodic classification of the elements and group separation of metallic cations), but the former is much more likely to be enduring and more likely to achieve general recognition. It is therefore adopted here for surfactants.

Most surfactants have a molecular structure that is essentially linear; one end is composed of a group of atoms having an affinity for water, termed hydrophilic, and the other end is antipathic to water and is termed hydrophobic. It is useful to introduce a third component of the molecule in the form of a "linking group." This group is absent from the simplest surfactants, but in many others it arises from considerations of manufacturing convenience. For example, from a suitable fatty material, such as oleic acid, to make a surfactant containing a sulphonate group, a simple method is condensation of the acid chloride with an hydroxy- or aminoalkylsulphonate—

$$R{\cdot}COCl + CH_3NH{\cdot}C_2H_4SO_3H \xrightarrow[-HCl]{} R{\cdot}CO{\cdot}N(CH_3)C_2H_4SO_3H$$

The components of the resulting surfactant are: oleic hydrophobic group, amide linking group and sulphonate hydrophilic group. Manufacturing convenience is not the sole justification for introducing a particular linking group; some groups are inserted to confer desirable physical properties on the product, *e.g.*, increased solubility, and others may modify the surface activity of the compound.

Most schemes of identification are introduced by an attempt to classify the types of surfactant likely to be encountered, but these all use a "nesting" sub-division, whereas the subject seems to demand a two-way, or even a three-way system, as described below. Only in this fashion can the present number of surfactant types (over 60 sub-classes of anionic surfactants are listed in one comprehensive scheme[5]) be classified in a manner useful to the analyst. In this review, it is possible to give only the main divisions of the groups (see Table I), but a few notes on the sub-division of some of the classes are added.

TABLE I

MAIN DIVISIONS OF GROUPS PRESENT IN SYNTHETIC DETERGENTS

HYDROPHOBIC GROUP:	LINKING GROUP:	HYDROPHILIC GROUP:
Naturally occurring acids, etc.—	*None*	*Anionic*—
Fatty acids (*a*)		Carboxylate
Fatty alcohols (*b*)	*Ether*—	Sulphate
Rosin acids	$-(O\cdot CH_2CH_2)_n-$	Sulphonate
Naphthenic acids	$-S(CH_2CH_2O)_n-$	Others
Hydrocarbons—	*Ester*—	*Cationic*—
Petroleum (1 ry)	$-CO\cdot O\cdot CH_2CH_2-$	Amine salts
Petroleum (2 ry)	$-CO\cdot O\cdot CH_2CHOH\cdot CH_2-$	Quaternary ammonium
Fischer - Tropsch	$-O\cdot CO\cdot CH_2-$	Quaternary heterocyclic
Synthetic alkyl (*c*)	$-O\cdot CO\cdot CH-$	Non-nitrogenous
Alkylbenzene	$-O\cdot CO\cdot CH_3$	
Alkylnaphthyl	Carbohydrate derivatives etc. (*d*)	*Non-ionic*—
Ethers—		Polyoxyethylene
Polyoxypropylene	*Amide*—	Polyhydric alcohol
	$-CO\cdot NH\cdot CH_2-$	*Ampholytic*—
	$-CO\cdot NH\cdot CH_2CH_2-$	Aminocarboxylic
	$-CO\cdot N(CH_3)\cdot CH_2-$	Aminosulphonic
	$-CO\cdot N(CH_3)\cdot CH_2CH_2-$ etc. (*e*)	

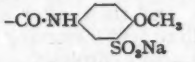
(*a*) This class is sub-divided into (*i*) "natural" mixtures recognisably derived from coconut oil, palm oil, tallow, etc., (*ii*) "technical" lauryl, ceto-stearyl, etc., which are fractions of the natural mixtures, and (*iii*) "pure" lauryl, oleyl, stearyl, etc., which, incidentally, may contain up to 20 or 30 per cent. of homologues.

(*b*) These may be naturally occurring alcohols or alcohols made by reducing fatty acids. The class can be sub-divided as in (*a*).

(*c*) This class contains compounds synthesised from shorter olefines, examples being nonanol and tetrapropylene.

(*d*) This class occurs in substances, such as sorbose and sorbitan, esterified with a hydrophobic group and allowed to react with ethylene oxide for hydrophilic properties.

(*e*) A large number of other amide groups are to be found, especially in conjunction with the sulphonate hydrophilic group, and two further examples are—



$-CO\cdot NH\cdot CH_2CH_2OCH_2SO_3Na$

Amide linkages are to be found almost exclusively with hydrophobic groups derived from fatty acids.

QUALITATIVE TESTS

One of the first systems of qualitative analysis of surfactants was published by Linsenmeier[6] in 1940. This, like many of the systems described in the following decade, consisted simply of a succession of empirical tests, and the scheme does not cover the many types of product now being marketed nor does it deal satisfactorily with mixtures. Of later systems, that of Kortland and Dammers[7] is one of the most useful; it deals with mixtures and incorporates the useful features of previously published schemes of identification.

HYDROPHILIC GROUPS—

The first step in the identification is to test for anionic, cationic and non-ionic groups, and in this connection the methods of Holness and Stone,[8] particularly the use of the quaternary test reagent Dimidium bromide, are worthy of study. If Dimidium bromide is not available, the required information can be obtained by an elaboration of the conventional tests (such as qualitative test (*vi*) in a previous publication[2]), acid methylene blue *plus* a trace of anionic surfactant being used to test for amines (cationic-active substances at pH 1 to 2) and alkaline bromophenol blue *plus* a trace of cationic surfactant to test for soaps; the blue colour is discharged from the lower organic layer in both tests. A substance that gives both reactions is an ampholytic surfactant.

For non-ionic surfactants, thermal decomposition as described by Rosen[9] is a useful test, for it will detect and distinguish oxypropylene as well as oxyethylene groups. The ceric nitrate test described by Karabinos, Kapella and Bartels[10] is useful for the polyhydric alcohol type of non-ionic surfactant.

NITROGEN—

If the sample contains nitrogen, it is desirable to determine whether this is present in an anion or cation, or in a non-ionic molecule. Distinction by precipitating a phosphonium-anionic compound and testing for nitrogen in this is described by Holness and Stone.[8]

Ion exchange is an alternative technique, and, in a laboratory equipped for convenient micro or semi-micro determination of elements, it is useful to determine nitrogen quantitatively, (*a*) in the sample, (*b*) in a portion after passage through a cation-exchange resin, and (*c*) in a portion after passage through a mixed-bed ion-exchange resin. The contents in each of the three ionic forms can then be calculated. On the subject of mixed-bed ion exchange, passage of a solution of the sample through such a column is a useful preliminary to qualitative tests for non-ionic surfactants, as numerous potential interferences are thereby removed.

ANIONIC SURFACTANTS—

If anionic surfactants are present, a quantitative or semi-quantitative determination of the proportions of various classes is useful, and a procedure slightly more elaborate than the Kortland and Dammers "pre-analysis" is recommended. Aliquots of a solution of the substance are heated under reflux with alkali and acid (*e.g.*, 30 minutes with *N* sodium hydroxide and 2 hours with 2 *N* sulphuric acid), and then the anionic surfactant in each and in the original solution is determined colorimetrically or volumetrically. The surfactant remaining after acid hydrolysis is probably alkylsulphonate or alkylarylsulphonate, the portion destroyed by acid hydrolysis, but stable to alkali, is probably a sulphated fatty alcohol and that hydrolysed in both acid and alkali is probably sulphated or sulphonated ester. Compounds with an amide linking group are partly hydrolysed in both acid and alkaline media, the degree of decomposition being to a large extent characteristic of the amide group, and, with standard conditions of hydrolysis, an experienced investigator can often recognise the particular amide from the ratios of the titrations or colorimeter readings.

SEPARATIONS—

The main identification scheme consists basically of a series of extractions, firstly of unsulphonated materials and additives, and then, after acidification, of fatty acids. The remaining mixture is then boiled with dilute acid (assuming that the preliminary tests showed some loss of anionic surface activity on acid hydrolysis) and fatty alcohols (from sulphated alcohols), fatty acids (from carboxylic esters) and alkylarylsulphonates are separated. For details, see the paper by Kortland and Dammers[7] and also the other schemes listed as references 1 to 8 of that paper.

MISCELLANEOUS TESTS—

A few further tests, especially a test for glycerol, and, if nitrogen is present, for primary and secondary amines, are usually sufficient to complete the classification of the surfactant. Ultra-violet spectrophotometry, as described by Reid, Alston and Young,[11] is useful for identifying alkylaryl compounds and also certain non-ionic and cationic surfactants. When a spectrophotometer is not available, the Guerbet and Echtrosalz tests are used to identify benzene and naphthalene derivatives. A few other useful and novel tests are described by Karabinos, Kapella and Bartels.[10]

In certain circumstances, for example, to distinguish a sulphated ester from a sulphonated ester, quantitative determinations (of sulphate before and after hydrolysis or of hydroxyl groups after hydrolysis) are useful. In fact, it is advantageous to apply quantitative considerations to most of the analytical operations in order to confirm the inferences that are made. Without quantitative tests, identification of non-ionic surfactants is virtually impossible as, within the main groups of esters, amides, phenol and alcohol derivatives, the compounds differ so little from one another. Suitable quantitative methods are described in a later section.

HYDROPHOBIC GROUPS—

Most of the published schemes of qualitative analysis terminate with the allocation of the surfactant to one of a number of classes and do not deal with characterisation of the hydrophobic group. A complete analysis requires this information, and, for a sulphate or

carboxylate ester, the fatty acid or alcohol isolated after hydrolysis can be analysed for acid or hydroxyl value, iodine value and setting point; from these results it may sometimes be possible to effect identification. Fractional distillation is rarely practicable owing to the smallness of the samples, but gas - liquid partition chromatography[12,13,14] is a valuable procedure. For other hydrophobic groups, chemical methods are of little use, but paper-chromatographic methods have been described for anionic,[15] cationic[16] and non-ionic[17] surfactants. Infra-red spectrophotometry[18] will also reveal the structure of the hydrophobic groups, and X-ray diffraction[19] may be useful.

Extraction methods

With regard to quantitative analysis, the majority of the determinations that are made in analysing detergents as raw materials, in process control and as finished products are extraction methods. Although there is little novelty in many of the methods, a review of the subject may be useful.

The principal extractants are (*a*) light petroleum, which dissolves unsulphonated hydrocarbons and unsulphated alcohols, (*b*) ethyl ether, which dissolves alkanolamides also, and (*c*) ethanol, which dissolves nearly all the organic constituents. The principal techniques are liquid - liquid extraction in separating funnels or, occasionally, in stoppered cylinders, and liquid - solid extractions in Soxhlet thimbles or with decantation through filter-paper.

Light petroleum—

Typical procedures for extractions with light petroleum are described in the British Pharmacopoeia[20] and United States Pharmacopoeia[21] monographs for sodium lauryl sulphate. The sample is dissolved in 50 per cent. ethanol, as the unsulphated alcohol is less soluble in this than in an aqueous solution of the sample (owing to reduced micellar effects) and emulsification difficulties are fewer, and then three extractions with petroleum are made. The combined extracts are dried, the solvent is removed by distillation and the residue is weighed. A certain proportion of free lauryl alcohol remains in the surfactant solution, as is shown by tests on synthetic mixtures; even after five or ten extractions. The loss depends on the concentration of surfactants, and, for reproducible results, the latter is arbitrarily fixed at about 5 per cent. w/v. Blank[22] describes a similar procedure for alkylarylsulphonates, and extraction with light petroleum is also used to determine soaps in admixtures with synthetic surfactants,[23] the aqueous alcoholic solution of the sample being first acidified to liberate the fatty acid.

Ethyl ether—

In extractions with ethyl ether, the optimum ethanolic content of the aqueous phase is less, often only 20 to 30 per cent., otherwise a clearly defined interface will be difficult to achieve. Ethyl ether is a better solvent than light petroleum for fatty materials, and, when its drawbacks of greater solubility for ethanol and hydrochloric acid are unimportant, it is to be preferred. In many applications, for example, in the qualitative analysis schemes,[7,8] a compromise with a mixture of light petroleum and ether is made. Incidentally, possible confusion with petroleum - ether mixtures is one reason why the description "petroleum ether" for the pure hydrocarbon should be abandoned.

Extraction of a built detergent with ether is used in determining alkanolamide and other additives, the percentage of free fatty matter found by extraction with light petroleum being deducted to give the additive content. Ethylene oxide derivatives with an average of two or three oxyethylene units will be only partially extracted and the higher ethoxylates will remain in the aqueous layer. A liquid - solid extraction of soap with dioxan has been described[24] as a method of dealing with the longer polyoxyethylene compounds, but for determining all additives, whether they be alkanolamides or polyoxyethylene derivatives, the ion-exchange method described later is best.

A very general use of extraction with ether is in determining the active ingredient of sulphated alcohols,[20,21] and analysis of sulphated esters is often made on similar lines. The sulphate groups are split off by acid hydrolysis and the resulting alcohol is extracted with ether, washed, dried, isolated and weighed. After deducting the percentage of unsulphated alcohol, found by extraction with light petroleum, the content of active constituent can be

calculated. Ethyl ether will also extract alkylarylsulphonates from concentrated ($> 4\ N$) hydrochloric acid solution, and analytical procedures based on this are described by House and Darragh.[25]

ALCOHOL—

Extraction with alcohol is used to determine the total organic content of built detergents,[23] and, by separately determining unsulphonated matter, additive, chloride, etc., the surfactant content can be found. The operation of taking the residue after a few extractions, dissolving it in a small amount of water and re-precipitating with alcohol is necessary to ensure extraction of small amounts of active material contained within the beads of spray-dried powders. The difficulty of extracting this fraction is one reason why the use of a Soxhlet apparatus is not generally to be recommended. A method described by Blank[22] omits dissolution in water, and the insoluble residue, not the extracted material, is weighed. Both these changes make for simplification in analysing non-spray-dried alkylbenzenesulphonates, but they may lead to errors in other applications.

In most contexts, "alcohol" implies ethanol, but the homologues have useful special applications. *iso*Propyl alcohol,[26] *tert.*-butyl alcohol[8] and *n*-butyl alcohol[27] have the advantage of permitting a liquid - liquid extraction procedure, which is immediately applicable to liquid detergents and to pastes, etc. As an example, the sample is dissolved in approximately 50 per cent. aqueous *iso*propyl alcohol and the mixture is saturated with sodium carbonate at about 40° C. Separation into two layers occurs, and a known aliquot of the alcohol layer can then be evaporated and the residue weighed to indicate the percentage of alcohol-soluble matter. This liquid - liquid extraction is a simple way of isolating the active constituent before qualitative analysis or for equivalent-weight determinations.

COLORIMETRIC DETERMINATION OF SURFACTANTS

After gravimetric procedures we pass on to colorimetric methods. These are conveniently dealt with before titrimetric analyses, because most methods of end-point detection depend on the use of the reagents that are employed in colorimetric work. The most widely used colorimetric method for anionic surfactants is that described by Jones,[28] which consists in shaking an aqueous solution of the sample with methylene blue (designated MB·Cl) and chloroform. The surface-active agent forms a salt that is soluble in chloroform, and the excess of methylene blue remains in the aqueous layer, *e.g.*,

$$\underset{\text{water-soluble}}{R\text{SO}_3\text{Na}} + \text{MB·Cl} = \underset{\text{chloroform-soluble}}{R\text{SO}_3\text{·MB}} + \text{NaCl}.$$

By spectrophotometric measurement of the blue chloroform extract or by comparison with standards, the surfactant content of the solution can be determined. Subsequent workers have tried to overcome interferences to which the method is subject, and prior separation by extraction with an amine[29] has been suggested; but simplest and best is the Longwell and Maniece method,[30] in which a preliminary extraction with methylene blue from alkaline solution is carried out. Methyl green has been claimed[31] to be better than methylene blue, but the workers made their comparison with an early modification[32] of Jones's method and not with the later Longwell and Maniece variant. Rosaniline[33] has also been used in the same manner as methylene blue.

Soaps and cationic surfactants could be considered outside the scope of "synthetic detergents," but their colorimetric determination can briefly be mentioned. The procedures are basically the same as for synthetic anionics, except for replacing methylene blue by reagents such as pinacyanol and bromophenol blue[34] and of appropriate pH control. Quaternary cationic surfactants can also be determined[35] by adding a known amount of anionic surfactant and determining the excess, preliminary trials having established the amount of anionic necessary to give a slight excess, by the methylene blue method.

For determining non-ionic surfactants, most colorimetric and volumetric methods are based on precipitation procedures and involve a conventional colorimetric or titrimetric determination of the inorganic reagent in the precipitate or filtrate. Such methods are described in a later section. Colorimetric procedures that do not require a separation by filtration or centrifugation are adaptations of colorimetric tests, details of a starch - iodine procedure[36] and a cobalt thiocyanate method[37] having been published.

THE ANIONIC - CATIONIC TITRATION

When present together in aqueous solutions, anionic and cationic surfactants will neutralise the surface activity of each other. This is the basis of an early technique of anionic - cationic titration, in which one species is determined by titrating with a standard solution of a surfactant of opposite type, neutralisation of surface activity being shown by a sharp rise in surface tension.[38] Another means of end-point detection was based on the colour change of an indicator or dye-stuff in the presence of long-chain quaternary compounds, and the change of bromophenol blue from purple to sky-blue was used by Hartley and Runnicles.[39]

An enhanced precision became possible when a two-phase titration procedure was introduced, both Epton[40,41] and Barr, Oliver and Stubbings[42] independently combining the idea of a titration with Jones's colorimetric extraction method. The basis of the titration was transformed from a surface-active effect to a matter of partition between aqueous and organic phases, and the end-point was denoted by the movement of an indicator ion from one phase to the other.

END-POINT DETECTION—

Methylene blue is often used as indicator, and with anionic surfactants it forms a chloroform-soluble salt according to the equation shown on p. 82. A cationic surfactant, added as titrant, preferentially forms a salt with the anionic surfactant, and when all the free anionic surfactant has reacted the titrant (designated CS·Br) begins to react with the methylene blue salt and displaces methylene blue back into the aqueous layer—

$$\underset{\text{chloroform-soluble}}{R\text{SO}_3\cdot\text{MB}} + \text{CS}\cdot\text{Br} = \underset{\text{water-soluble}}{R\text{SO}_3\cdot\text{CS}} + \text{MB}\cdot\text{Br}.$$

The end-point may arbitrarily be taken as (*a*) the first appearance of blue colour in the aqueous layer, (*b*) the complete transfer of blue colour to the aqueous layer, or (*c*) partial transfer to the appearance of equal colour intensities in the two layers.

The titration may also be done in the reverse manner, adding the indicator to the cationic surfactant in the titration vessel and titrating with an anionic surfactant. Method (*c*) of detecting the end-point is again the most usual with methylene blue, but two other methods are (*d*) the first appearance of blue colour in the chloroform layer, and (*e*) the complete transfer of blue colour to the chloroform layer (personal communication from D. C. Cullum).

An anionic indicator can similarly be used, the role of cationic and anionic surfactants being interchanged, and bromophenol blue[42] has b widely used. Here, method (*d*) seems to be the usual choice of end-point, (*b*) has been used,[43] but (*e*) is probably best.

BLANK CORRECTIONS—

At the end of the titration for end-points (*a*) and (*e*), the whole of the indicator is in combination with surfactant, and a blank correction, which is constant, calculable, reproducible and readily determined, must be applied. At end-point (*c*), only part of the indicator remains combined with surfactant and the necessary correction depends upon the proportion involved; this, in turn, depends upon the relative volumes of the aqueous and chloroform layers. Experimental determinations of the blank or of a correction factor have been described by several workers,[25,44] and most others recommend closely similar conditions for standardisation and for test in order to overcome the effect of the blank correction. The subtraction of a blank correction in one method[22] appears to be based on a misunderstanding of the principle of the titration, but as similar conditions are used for standardisation no error will result.

Epton ignored the correction, for with end-point (*c*) he found that a cationic titrant standardised by the dichromate method gave correct results, but it is probable that the neglected indicator blank compensates for errors in the dichromate standardisation. Barr, Oliver and Stubbings and other workers used end-points (*b*) (for methylene blue) and (*d*) (for bromophenol blue) in which no blank correction is applicable, and standardising the cationic titrant against anionic surfactants of known composition gave accurate results.

STANDARDISATION—

Whatever the end-point, it seems desirable to standardise the cationic titrant against a pure known anionic compound, *i.e.*, a substance whose equivalent weight is known or has been accurately determined and which has been analysed for water, sodium sulphate, etc., all these being minor impurities.

Many workers follow Epton and carry out titrations of about 10 ml with 0·004 or 0·005 *M* titrant, but larger titrations with more dilute solutions, *e.g.*, about 20 ml of 0·001 *M* titrant, as used by Barr, Oliver and Stubbings,[42] normally give more precise results and are at least as accurate if due regard is paid to the blank value.

All titration procedures are equally suitable for determining anionic or cationic surfactants, the concentration of the other being known, and it is also immaterial whether the solution of unknown concentration is placed in the titration vessel or in the burette, although the former is usually the more convenient.

STOICHEIOMETRY—

The accuracy of the titration depends on the titrant reacting with the whole of the anionic surfactant (taking, for example, the use of a cationic titrant with bromophenol blue as indicator) before reacting with the indicator. This cannot always be realised with commercial materials and often the indicator partially reacts before the surfactant molecules of shorter chains have completely combined. An error is thereby caused and this is liable to be greatest if end-point (*d*)—the first appearance of blue colour in the chloroform—is used. The error is minimised in Cullum's procedure, method (*e*), of titrating to complete transference of the indicator to the chloroform layer, and this gives the most nearly stoicheiometric reaction of titrant with commercial surfactants.

OTHER INDICATORS—

The indicators mentioned are generally suitable only for certain pH ranges—in acid media for methylene blue and in neutral or slightly alkaline solution for bromophenol blue—and other indicators have found application at different pH values or as alternatives within these ranges. Typical examples are eosin,[45] dichlorofluorescein[46] and Dimidium bromide. The last is described as a qualitative test,[8] but it can replace methylene blue for titrations in media of a wide pH range, especially with end-point (*e*).

OTHER METHODS FOR ANIONIC SURFACTANTS

Another volumetric method of analysis is also based on the formation of a chloroform-soluble surfactant salt; the titrant is not another surfactant, but simply 0·1 *N* sodium hydroxide. The method[47,48] consists in adding *p*-toluidine hydrochloride to a neutral aqueous solution of the anionic surfactant and extracting the resulting salt with chloroform. The chloroform extracts are then diluted with ethanol and the amine component is titrated with standard alkali. A gravimetric finish can be effected by evaporating the chloroform extracts to dryness and weighing the residue; a combination of gravimetric and volumetric methods will indicate the equivalent weight of the surfactant. Similar methods with benzidine in place of *p*-toluidine and precipitation instead of extraction have also been described.[22,49]

Quantitative acid hydrolysis has been used for analysing sulphated alcohols. The sample is heated for several hours with a known amount of 0·5 to 2 *N* hydrochloric, sulphuric or phosphoric acid and the increase in acidity is finally determined by titration with alkali.

$$ROSO_3Na + HCl + H_2O = ROH + NaCl + H_2SO_4.$$

There is a tendency for the results to be low, as hydrolysis is often incomplete even after 4 or 6 hours' boiling,[50] and there is also evidence that side reactions can occur and cause a slight decrease in the amount of acid liberated.

Extraction and weighing the alcohol produced by acid hydrolysis is described in an earlier section. This is not subject to the two objections just mentioned, first because more concentrated acid can be used without any loss of accuracy, and secondly, because any unattacked surfactant or product of a side reaction is weighed with the residue, which reduces the error and, incidentally, makes it positive instead of negative.

Another method is to determine the sulphate present after hydrolysis with hydrochloric acid and to correct for any sulphate initially present. This gives results more accurate than those by alkali titration, but it is not so reliable as the extraction method.

Determination of cationic surfactants

Colorimetric methods were indicated in a previous section, and, for titrimetric determination, the anionic - cationic titration has been dealt with in detail. A good review of gravimetric methods is included in a paper by Chinnick and Lincoln,[51] and the phosphotungstate method[52] is recommended.

Non-ionic surfactants

Two main applications to be considered are determination of non-ionic foam promoters in built detergents whose main constituents are anionic surfactant and condensed phosphate and determination of anything from 0 to 100 per cent. of non-ionic surfactant in liquid detergents.

The earliest methods of analysis simply equated the content of non-ionic constituents to the difference between alcohol-soluble matter and ionic-surfactant content. In certain applications this method is quite adequate, but no further comment is needed here, as the two groups of constituents are dealt with in other sections. Slightly more specific is extraction with ethyl ether of non-ionic additives from an aqueous or aqueous alcoholic solution of the sample, as described earlier, but this fails for most ethylene oxide derivatives.

Ion-exchange methods—

A more general method, equally applicable to alkanolamides and polyoxyethylene derivatives of any chain length is based on the use of ion-exchange resins. Weeks, Ginn and Baker[53] pass an ethanolic extract of the sample successively through separate columns of anion and cation-exchange resins in, respectively, the (OH) and (H) forms, whereas Rosen[54] uses only an anion-exchange resin in the chloride form and removes sodium chloride (presumably the method is inapplicable to amine-neutralised surfactants) by a subsequent extraction.

Better than either of these procedures is the use of a single column of a mixed-bed resin such as Amberlite MB1 or Biodeminrolit. The experimental procedure can be far simpler than any published method that I have seen, for neither dehydration of the sample nor separation of inorganic salts in a preliminary step is necessary. Built powder detergents are dissolved in a small volume of water (*e.g.*, 5 g in 50 ml) and an equal volume of alcohol (methanol, ethanol or *iso*propyl alcohol seem almost equally suitable) is added. The mixture is then transferred to the ion-exchange column (about 40 g of resin) via a filter consisting of a plug of cotton-wool. Other detergents can be dissolved directly in 90 per cent. *iso*propyl alcohol and passed into the column; 90 per cent. *iso*propyl alcohol is invariably a useful solvent for washing the non-ionic material through. The combined effluent is evaporated to dryness and the non-ionic material is weighed.

The weighed non-ionic fraction, whether obtained by ether extraction or ion exchange, may consist of three groups of constituents: unsulphonated alkylate or alcohol, termed free fatty matter, alkanolamide additive and ethylene oxide derivatives. The first may be determined by extraction with light petroleum from aqueous alcoholic solution, the second by alkali or acid hydrolysis and isolation of the fatty acid and identification of the amine, and the ethylene oxide derivative by difference (only in part after ether extraction) or as described below.

Precipitation methods—

For determining ethylene oxide derivatives, several precipitation methods are available. The procedures can sometimes be applied to the original samples, but, by a preliminary ion exchange, interference from ions such as quaternary cationic compounds on one hand and sulphate on the other can be avoided. The precipitants generally used are ferrocyanide,[55] and, in conjunction with barium, molybdophosphate,[56] phosphotungstate[52] and silicotungstate.[57] The precipitates have a reproducible but not stoicheiometric composition, and an empirical gravimetric factor is needed for the particular compound being determined.

Alternatives to weighing the precipitate are volumetric or colorimetric determination of excess of precipitant in the filtrate[58] (generally used for ferrocyanide) and the colorimetric determination of the molybdate or molybdate complex[57,59,60] in the dissolved precipitate.

In all the foregoing precipitation methods, it is necessary to know the identity of the non-ionic surfactant, and, moreover, to have precise data relating to the type of surfactant

present in order to calculate its content. However, the methods can also be used as an aid in identifying the surfactant if its content is known, a special instance being to start with 100 per cent. material after an ion-exchange separation. The same remarks apply to the less specific methods of nitrogen content (of amides), saponification value (of esters), hydroxyl value and polyethanoxy content as determined with hydriodic acid.[61] When the identity of the surfactant is known, these determinations indicate its content; when the content is known, these determinations indicate something about its identity.

CLOUD-POINT—

For identification purposes only, the cloud-point can be used. This is the temperature at which a dilute aqueous solution of the compound becomes cloudy when heated. Although directly applicable to ethylene oxide compounds in the detergent range, it can be applied to the more highly ethoxylated wetting agents by using 5 per cent. aqueous sodium chloride as the solvent, and it can be applied to certain insoluble ethoxylate emulsifiers or foam promoters by using 15 per cent. aqueous ethanol as solvent, or, in addition, by blending the unknown surfactant with half its weight of a soluble ethoxylate.

The cloud-point indicates the balance between hydrophilic and hydrophobic properties in the molecule, and, for each hydrophobic group, a graph can be drawn relating the cloud-point and the number of ethylene oxide groups. When the cloud-point of a non-ionic surfactant has been determined, reference can be made to the series of graphs to determine the size of either the hydrophilic or hydrophobic group if the other is known or if their combined molecular weight is known. Certain hydrophobe - lipophobe titration procedures[62,63,64,65] can be used in the same way, and the density - cloud-point relationship[65] gives further information about an unknown non-ionic surfactant.

INORGANIC CONSTITUENTS

Sodium carbonate, as is indicated by the popular name "washing soda," is a well known inorganic detergent, and sodium salts of other weak acids—borate, silicate and phosphate—and sodium hydroxide itself are also used as detergents. Methods of analysing these materials are described in several textbooks and numerous papers, and except for phosphates a single reference[66] should suffice.

The analytical chemistry of the phosphates is complicated by the existence of a number of different compounds. Satisfactory methods of analysing these have been devised only in the last few years, and older texts contain misleading and inaccurate statements. It was the wide introduction of pentasodium triphosphate (more usually "tripolyphosphate" in the U.K.) that focused attention on the need for better characterisation of the condensed phosphates, and numerous precipitation methods have been described. For samples in which triphosphate predominates, the use of tris(ethylenediamine)cobaltic chloride as a precipitant[67,68] is far preferable to methods in which salts of manganese[69] or zinc[70,71,72,73] are used; the simple salts are of use in other tests when direct precipitation of pyrophosphate may be desired.

So far the inorganic compounds have been treated as separate constituents, whereas in built detergents they are part of a complex mixture. Analysis from this standpoint has been reviewed by Harris,[74] and brief notes are given below, some miscellaneous organic determinations also being described. The most general methods have been selected in preference to certain more rapid procedures that are of limited application.

SODIUM PHOSPHATES—

The alcohol-insoluble constituents are separated, dissolved in water and diluted to a known volume. Ortho-, pyro-, tripoly- and total phosphate are determined on separate aliquots by the methods indicated earlier. Possible interference of silicate, hydrogen peroxide or borate must be considered. Silicate and peroxide are readily removed by filtration and reduction, respectively, whereas borate will invalidate most of the simple titration methods, but not the titration of a molybdophosphate precipitate.

SODIUM SILICATE—

The sample is ashed, the residue is fused with potassium and sodium carbonate and the analysis is completed by the usual methods for silicates. More rapid methods can be used

with many materials, but a fusion step is often needed for accurate results, as condensed phosphates are otherwise liable to interfere. The usual hydrofluoric acid treatment is of little value when the precipitate is contaminated by sodium phosphate or sulphate.

SODIUM SULPHATE—

Gravimetric determination as barium sulphate is a simple and accurate method. Organic compounds can be removed by alcohol extraction either with ethanol from the dry solid or with a higher alcohol, alone or with ethyl ether, from an aqueous salt solution.

The presence of phosphates and perborate generally invalidates or complicates the rapid volumetric methods that have been described in the literature, although these are useful for certain materials.

SODIUM CHLORIDE—

Titration with silver nitrate potentiometrically, or with a chromate as indicator, in presence of calcium carbonate is usually satisfactory. Sodium chloride is slightly soluble in alcohol and this solvent cannot be used to separate the organic components.

SODIUM PERBORATE—

The peroxide content can usually be determined by dissolving the sample in dilute sulphuric acid and titrating with potassium permanganate. Borate is determined alkalimetrically with mannitol or glycerol.[75]

SODIUM CARBONATE—

Treatment of the sample with acid liberates carbon dioxide, which is absorbed in soda asbestos and weighed.

ETHYLENEDIAMINETETRA-ACETIC ACID—

A method based on colorimetric determination of the amount of nickel complexed by the ethylenediaminetetra-acetic acid has been described by Darbey.[76]

MAGNESIUM SULPHATE—

Magnesium, and indeed other metallic cations, can readily be determined by ordinary analytical methods after the sample has been ashed and the residue dissolved in dilute acid. The possible interference of phosphates must be borne in mind.

CARBOXYMETHYLCELLULOSE—

Colorimetric methods are most useful for determining cellulose derivatives in detergents, and procedures in which anthrone[77] and dihydroxynaphthalene[78] are used have been described. The degree of substitution of carboxymethylcellulose must be known in order to calculate the content, but this can often be estimated after applying both the foregoing methods.

FLUORESCENT DYE, PIGMENT AND PERFUME—

These components are determined by their obvious physical properties, rarely by chemical methods.

ABRASIVE—

The content is usually equated to the insoluble matter.

GLYCEROL—

The periodate method is applicable after removal of interfering constituents by ion exchange and solvent extraction.

TOLUENE OR XYLENE SULPHONATES—

These solubilisers can be determined by ultra-violet spectrophotometry[25] after ether extraction of long-chain sulphonates from the hydrochloric acid solution.

UREA—

Urea is usually present as solubiliser in liquid detergents that are free from amine cations, and it can then be determined from the difference between total nitrogen content and nitrogen present after passage through a cation-exchange resin. Titrimetric or gas-volumetric determination with hypobromite or bromate may be useful, ion exchange again serving to separate urea from possible interferences.

WATER AND ALCOHOLS—

The distillation method[75] is generally applicable, xylene being preferred as entrainer, as it readily removes the water (and peroxide) of crystallisation from phosphates and perborate. The liquid in the receiver can be tested for volatile alcohols (ethanol and *iso*propyl alcohol) and analysed quantitatively by the Karl Fischer method or colorimetrically with ceric nitrate.[79,80]

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Zone Electrophoresis

Some Basic Considerations in Design*

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Zone electrophoresis is differentiated from free-boundary electrophoresis. Some basic deseridata in electrode design and buffer stabilisation are discussed. The common matrices used are contrasted and their limitations are outlined.

ELECTROPHORETIC techniques in which paper, starch, agar and other separate media are used have been reviewed by Tiselius and Flodin[1] and have been called zone electrophoresis. The method is a relatively simple means of analysing mixtures of complex polyvalent ions in solution. Zone electrophoresis includes all methods that produce more or less completely differentiated zones of the individual components being separated. In this respect it can be distinguished from the free-boundary electrophoresis described by Tiselius,[2] although even by this technique, if two species differ sufficiently in their mobility, complete separation is achieved.

The relative simplicity of apparatus required to produce some degree of separation by zone electrophoresis has permitted workers to build their own instruments incorporating modifications specifically suited to their purses or to their specific requirements. The lack of conformity in design has made the direct comparison of results from different laboratories difficult. It has to be confessed, moreover, that some designs transgress the basic requirements for stable reproducible conditions.

Zone electrophoresis always requires some stabilising medium on which or in which lie the solutions to be analysed. A variety of materials have been used successfully; few of them are "inert" and few of them are well defined chemically. It is not surprising, therefore, that discrepancies sometimes occur between results obtained with successive batches of such a

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stabilising medium. Because filter-paper of one form or another is the most commonly used stabilising medium, the problems associated with its use will be given as a general example, although it must be remembered that each stabilising medium has its special advantages and disadvantages.

Reproducible electrophoretic analysis, whatever the technique, depends on the ability of the worker to achieve reproducible controlled conditions in successive analyses. Some general requirements deserve emphasis and are dealt with below.

Stable electrical conditions across the field of analysis

For most purposes, demands range from 50 to 300 volts per 20 cm strip length, and it is not difficult to arrange a constant-voltage supply through some simple form of mains rectifier or by using high-tension batteries. If higher voltages are required, as in the techniques for the separation of amino acids and peptides, special problems arise in insulation and in the dissipation of the heat generated.

Electrodes—

It is not possible to maintain satisfactory working conditions unless the design incorporates suitable electrodes properly housed.

Carbon electrodes polarise readily and tend to disintegrate with use.

Platinum foil or wire is not suitable for the closed electrode systems used for accurate mobility measurements, because of the evolution of gas on the surface of the foil. Since zone electrophoresis is rarely carried out in a closed system, platinum is suitable for the majority of designs.

Silver - silver chloride electrodes are satisfactory provided they are of adequate surface area; 60 sq. cm are ample to deal with most currents required in routine work.

Electrode housing—

The products of electrolysis arising at the surface of the electrode may interfere with the substances being investigated or disturb the buffer equilibria with consequent zone deformation. The early apparatus of Hardy[3] and Burton[4] suffered from this defect. Michaelis[5] pointed out the advantages of a separate electrode vessel suitably linked by an agar bridge or some other means to the main buffer compartment.

Whatever the form of the electrode, it is wise to house it in a compartment separate from that into which hang the ends of the paper strip on which the analysis is to take place.

Svensson[6] has stressed the consequence of the variations in density of buffer ions liberated as a result of electrolysis. In general, with the exception of ammonia, the acid radicle generated is lighter than the buffer and the alkali is heavier. There is a tendency to layering of the respective ions during the passage of current, and if steps are not taken to avoid this effect or to ensure that the junction with the main buffer compartment does not feed directly from the top or bottom of the electrode compartment, the layering may upset buffer equilibrium. Svensson also suggested the use of acid and alkali locks to overcome the difficulty, and Valmet and Svensson[7] have designed an electrode suitable for zone electrophoresis in which these hazards are guarded against.

Maintenance of buffer stability during analysis

Careful electrode design will obviate one of the causes of disturbance of buffer. The reservoirs should, nevertheless, hold sufficient bulk of buffer solution to limit pH changes from migration of ions other than those being investigated and to swamp inherent buffering effects of the ions being investigated. Proteins themselves are powerful buffers.

In the classical apparatus for free-boundary electrophoresis in which 10 ml of material are used for each analysis, the buffer chambers each have a capacity of 1000 ml. In zone electrophoresis the minimal safe ratio is probably 10 to 1 and preferably 15 to 1. Therefore, if the saturated filter-paper strip holds 20 ml of buffer solution, each buffer reservoir should hold 300 ml. The minimal safe ratio varies with buffer pattern. The buffer reservoir is assumed to be coupled through a lock to the electrode vessel, the capacity of which is not included in this calculation.

The buffer concentration selected for an analysis depends on individual requirements. High concentrations reduce mobility of the solute investigated, but limit diffusion and so produce sharper and more closely packed zones.

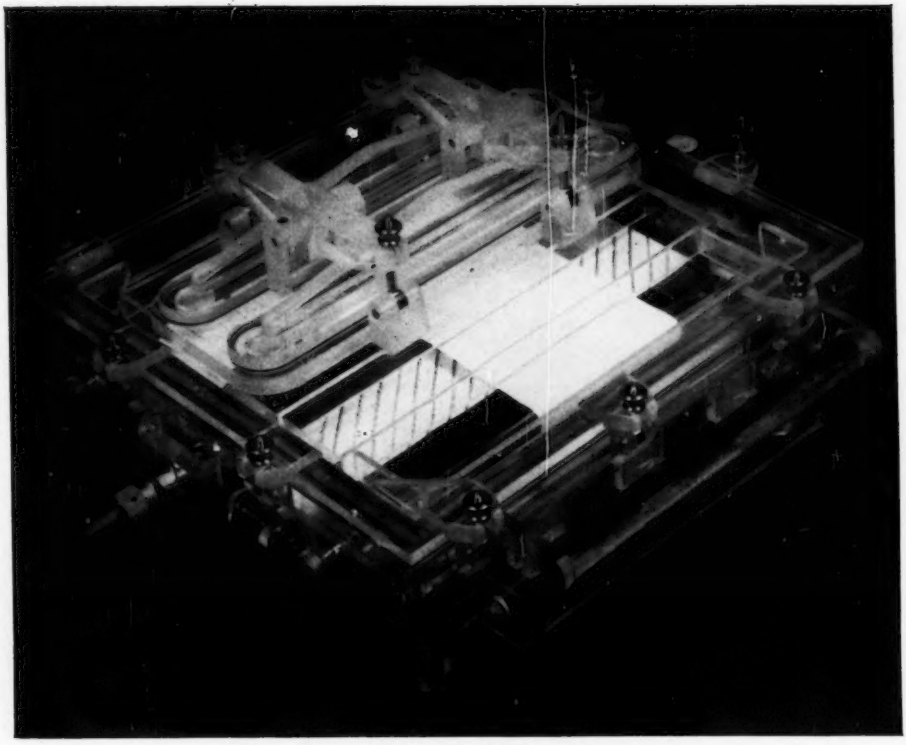

Fig. 1. Electrophoretic cell with lid fixed in position.

In position on top are the ports for applying specimens, and, through the Perspex lid, the analytical strip can be seen, the upper equilibrating strip having been removed

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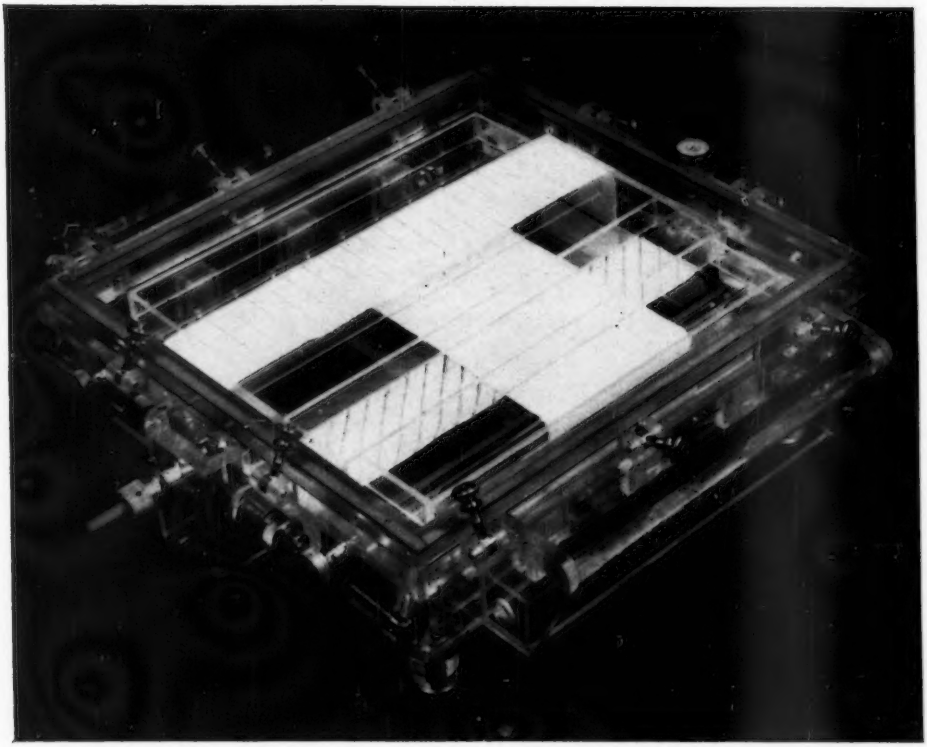


Fig. 2. Electrophoretic cell with lid removed; cut-away strips are placed in the position of the equilibrating strips.

On the left is the gearing for maintaining paper tension and in front and to the right is the electrode housing

In general, the smaller the molecular species analysed, the greater the risk of diffusion with consequent loss of definition. Since the diffusion increases with the time taken to complete the separation, it is customary to carry out the analysis of small molecules, such as peptides, over short periods of time at high voltages in apparatus specially designed to disperse the heat generated.

Stabilisation and formation of starting boundaries

Before the electrophoretic separation is started, it is essential to establish equilibrium between all compartments and between them and the stabilising material being used in the separation.

To this end we leave our apparatus sealed with the filter-paper strip in position and the buffer chambers fully charged for not less than 2 hours before placing the material to be analysed on the paper strip. To minimise disturbances, the material is applied to the strip through a small port, which is then immediately sealed.

For quantitative measurements, the material is applied with a micro syringe, but, for qualitative analysis, a simple applicator suggested by Dr. R. A. Kekwick is satisfactory. This is prepared by bending a short piece of thick (3 MM) filter-paper round the end of a 3-inch × 1-inch glass microscope slide and securing it with an elastic band. The secured filter-paper is then first saturated with the material to be analysed and subsequently pressed on the analytical strip and left there for a short time to allow exchange of the absorbed solution. During this period, the glass slide projects through the port and partly closes it. The applicator is subsequently removed and the port is sealed completely.

Temperature control

Gross fluctuations of temperature during analysis will disturb the formation of clearly defined zones. These may result from inadequate insulation of the apparatus or from the use of currents of too high a density. In the apparatus described by Franglen, Martin and Treherne,[8] direct measurements with thermocouples showed that temperature fluctuations were less than 0·3° C over 16 hours at 19° C during an analysis conducted at 5 mA per 20 cm strip width and 100 volts per 23 cm strip length. Fluctuations of this order produced no appreciable zone disturbances. Potential gradients as high as 10 volts per cm were applied to the paper without serious temperature increases.

The matrix and the maintenance of buffer equilibrium throughout its substance

In free-boundary electrophoresis, boundary disturbances arising from thermal diffusion and density gradients are reduced by maintaining the temperature of the whole analytical cell and the buffer chambers at 4° C. This inevitably increases the expense and unwieldiness of the apparatus.

In zone electrophoresis, the presence of the supporting medium largely avoids convections from density gradients, and, provided current density is not too great, heat will be dissipated to the surrounding media without serious disturbance of zones.

One of the major problems of design in apparatus is to ensure the maintenance of even buffer concentration throughout the analytical strip.

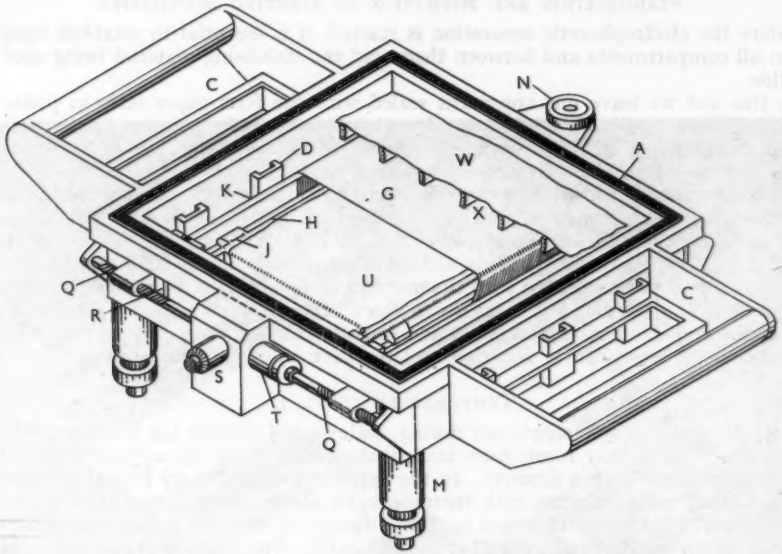
With apparatus in which the analytical strip is suspended as an inverted V above the buffer chambers, the amount of "wetness" (which determines the amount of buffer) each square of the paper will retain varies inversely with its height above the levels of liquid in the buffer chambers.

It is, therefore, extremely difficult, if not impossible, to maintain comparable conditions of equilibrium over all parts of the strip.

If, in addition, the design involves a large dead space around the paper strip, it will be difficult to avoid a distillation effect from the strip into the surrounding atmosphere, as even at the lowest current densities there must be a thermal gradient between the surface of the paper and the surrounding atmosphere. This is true for any design in which an exposed analytical strip is used. The apparatus must be adequately sealed to prevent the escape of water vapour, which may produce distortion of the separation.

Many designs in which the analytical strip lies horizontally in the chamber suffer through lack of a means of preserving an even paper tension. In these designs, as the cellulose fibres

become soaked with buffer they stretch and the strip sags with consequent pooling of buffer solution in the centre. In the apparatus we developed (see Figs. 1, 2 and 3), adjustable tension bars permit the slack to be taken up at will, and the atmosphere immediately surrounding the paper is kept saturated by two independent sheets of thick filter-paper placed above and below the working strip and saturated from independent buffer chambers.



A = Rubber seating
C = Electrode housing
D = Agar bridge connecting electrode housing with buffer resevoir
G = Analytical strip
H = Clamp for analytical strip
J = Retaining tongue for clamp
K = Glass rod over which analytical strip passes
M = Adjustable levelling legs
N = Spirit level
Q = Gear mechanism for maintaining paper tension
R = Spring-loaded plate
S = Guarded control knob for gear
T = Clutch for gear
U = Lower equilibrating paper
W = Upper equilibrating paper
X = Grid for supporting upper equilibrating paper

Fig. 3. Electrophoretic cell (lid bolts not shown). Reproduced by permission of the Editor from *J. Clin. Path.*, 1955, 8, 144

The stabilising media and their effect on the separation of mixtures

In 1939, Coolidge[9] described the use of glass-wool as a stabilising medium in zone electrophoresis. In the last 10 years, the successful use of numerous materials ranging from filter-paper of almost every texture to latex foam[10] has been reported.

Many of the materials used are not definable chemical entities, and reproducibility of analysis depends therefore on careful standardisation of preparative procedures. Five materials are widely used at present. They are filter-paper, either in the form of sheet or pulp, cellulose acetate,[11] starch grain,[12] starch gel[13] and agar gel.[14]

Their potential water content ranges from 98·5 per cent. for agar gel and 85 per cent. for starch gel down to the order of 25 per cent. for starch grain and as low as 10 per cent. for cellulose acetate. It might therefore be assumed that agar and starch gel most nearly approach the classical technique. This is not in fact true; the extremely fine lattice of the starch gel appears to produce a "sieve" effect, so that the order of separation is a function of size and shape of the molecule as well as of its charge. Indeed, Franglen and Gosselin[15] have shown that, under prescribed conditions, starch gel will demonstrate the polymerisation of structurally well defined dyes, such as bromocresol green.

These observations stress the care with which one must assess the description of "new" components separated by starch gel techniques.

Filter-paper, even of the finest grades, is not an inert material. In addition to the free hydroxyl groups, which give it its hydrophilic qualities, in the process of bleaching it acquires some free carboxyl groups. Normally, therefore, it carries a net negative charge, and the solvent will move across it in the direction of the current; the endosmotic flow. This effect is much more pronounced at low ionic concentrations and with "dry" supporting media.

Jermyn and Thomas[16] have attempted to overcome this effect by pre-treatment of the paper, and Porath[17] has attempted to neutralise the effect by a hydrodynamic balance. Whatever approach is used, it is important that endosmotic flow be controlled.

The charged radicles on the paper may result in the binding of materials being analysed to its surface. Hence, in the analysis of serum proteins, natural albumin will form a "carpet" on the surface of the filter-paper behind the free moving zone, so contaminating the zones of slower moving globulins migrating behind it.

The selection of buffers

A wide range of buffers can be used in zone electrophoresis, although, at pH values above 10, filter-paper becomes so fragile that it is difficult to maintain an adequate tension.

If the design of apparatus results in marked evaporation from the surface of the stabilising medium, buffers containing volatile constituents should be avoided; indeed, the addition of glycerol to the buffer has been recommended by some workers to reduce the extent of evaporation.

The ionic pattern of the buffer may be modified to achieve improvement in the separation and definition of specific components in a mixture. Consden and Stainer[18] have developed the use of borate buffers for the separation of sugars by the formation of a charged borate couple, and Aronsson and Grönwall[19] have experimented with the use of trisethylene-diaminetetra-acetic acid buffer for the detailed separation of proteins. This field is in its infancy and there will most certainly be rapid developments in the future.

The identification and analysis of components after separation

The identification of coloured substances, such as haemoglobin, presents little difficulty. Ultra-violet light may be used as a supplement to normal light.

The commonest general technique of identification is the coupling of the separated component with acid-wool or indicator dyes or by reactions such as the periodic Schiff reaction for glyco proteins.[20] These reactions are not always stoicheiometric and the precise quantitative analysis of mixtures by zone electrophoresis still remains a complex problem.[21]

The introduction of the agar gel technique by Grabar and Williams[14] was designed to allow the immunological behaviour of individual proteins and protein conjugates to be studied. This is an extremely delicate technique, but it must be realised that, in the analysis of large molecules, fluctuations in immunological behaviour may be the consequence of minute changes in the pattern of surface charge not easily correlated with the chemical characteristics.

It is evident from what has been said that the potential of zone electrophoresis as an analytical tool has not yet been fully exploited. It is equally evident that such exploitation can only be successful if the basic principles of design are not transgressed.

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The Determination of α-(4-Chloro-2-methylphenoxy)-propionic Acid in Commercial Acid of this Name

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A method is described for the determination of α-(4-chloro-2-methylphenoxy)propionic acid in the presence of α-(4:6-dichloro-2-methylphenoxy)-propionic acid, α-(6-chloro-2-methylphenoxy)propionic acid and α-(2-methylphenoxy)propionic acid by chromatographic separation of the active acid and its subsequent measurement by ultra-violet absorption. The chromatographic separation is similar to that used by Freeman and Gardner in the examination of chloromethylphenoxyacetic acid.

To improve the accuracy of the method, the ultra-violet absorption of the main band is measured against a known similar concentration of 4-chloro-2-methylphenoxypropionic acid; in this way, a small difference in ultra-violet absorption can be measured and a more precise reading can be made with a spectrophotometer.

Some of the substituted β-phenoxypropionic acids, which are known to be present in a few chloromethylphenoxypropionic acid formulations, have been examined; these were found to have similar ultra-violet absorption characteristics to the α-analogues. β-(4-Chloro-2-methylphenoxy)propionic acid and β-(4:6-dichloro-2-methylphenoxy)propionic acid do not interfere, as they are eluted from the column before any α-acids appear in the eluate.

Selective weedkillers containing α-(4-chloro-2-methylphenoxy)propionic acid as active constituent have recently been introduced. Technical chloromethylphenoxypropionic acid (CMPP), from which the formulations are prepared, is manufactured by processes similar to those used to prepare chloromethylphenoxyacetic acid (MCPA), and, apart from the active constituent, will be found to contain α-(6-chloro-2-methyl)-, α-(4:6-dichloro-2-methyl)- and α-(2-methylphenoxy)propionic acids. If the α-chloropropionic acid used in the manufacturing process contains any of the β-isomer, a small portion of the corresponding β-acids can also be expected in the final product.

It must be noted that α-(4-chloro-2-methylphenoxy)propionic acid can exist in two optically active forms, only one of which has herbicidal properties; however, since no change in the ratio of D to L isomers can occur under normal conditions of manufacture, a determination of DL-(4-chloro-2-methylphenoxy)propionic acid can be regarded as a measure of biological activity.

The determination of the amount of active acid present in technical CMPP may seem a problem analogous to the analysis of MCPA, and similar methods to this end were first considered, *i.e.*, differential refractometry, infra-red and ultra-violet spectrophotometry and liquid - liquid partition chromatography.

Differential refractometry, although it gave satisfactory results for synthetic mixtures of the four principal acids, gave low results with most commercial acids. The cause of this trouble was found to be the presence of small amounts of tarry material in the extracted

carboxylic acids; this tar was non-volatile when the acids were heated *in vacuo* at 150° C, and its strong interference with the measurement of mixtures of synthetic acids by this means was also noted. Direct measurement of the active acid by ultra-violet absorption was ruled out by the strong interference of several of the major impurities.

An infra-red absorption method has been developed, but, in our opinion, its accuracy is not at present as good as that of the proposed method.

A gas-chromatographic method has also been examined. In this technique, a portion of the extracted acids was esterified with methanol. The yield of ester was about 95 per cent. of the theoretical, and it was estimated that all the acids were esterified to the same extent. A small volume of the resulting mixture of esters was examined by using a 6-foot column of 35 to 80-mesh Chromosorb containing 20 per cent. of the sodium salt of dodecyl benzenesulphonic acid as static phase and heating at 205° C. Two methods of measurement were possible, a direct determination of the 4-chloro-2-methyl acid could be made by measuring the height or area of the main elution peak, or, by placing a larger sample on the column, the percentage of each impurity detected could be measured and the sum of these figures subtracted from 100. Both methods of measurement were liable to error, either because unidentified acids having the same retention time as the main component were present or because impurities were not detected by the column conditions used. This meant that the result, although it was often correct, had, in the absence of supporting evidence, to be regarded as a maximum figure only. This reservation was subsequently borne out by experience.

The liquid - liquid chromatographic method described by Freeman and Gardner,[1] which was later adopted by the Joint Herbicides Committee of the Association of British Insecticide Manufacturers and the Ministry of Agriculture, Fisheries and Food, was tried on a mixture of the principal CMPP acids. The components of the mixture could not be separated, but it became evident that some degree of separation would be possible if the column conditions were modified. Such modifications were therefore examined in more detail.

Experimental

Samples of the pure α and β mono and dichloro acids were prepared, and the purity of each was checked by gas chromatography of its methyl ester. Ultra-violet measurements were made in 4-cm silica cells with a Unicam SP500 spectrophotometer.

An attempt (based on Freeman and Gardner's work[1] on MCPA) was made to separate the mixed acids on a chromatographic column.

With a 0·25 *M* aqueous phosphate buffer supported on kieselguhr as static phase and an equilibrated diethyl ether - chloroform mixture as moving phase, it was found that α-(2-methylphenoxy)propionic acid was easily separated from the other main constituents, as it was more soluble in the static phase and was therefore retained on the column for a longer time. It was observed that, whereas chloroform tended to separate the 4:6-dichloro-2-methyl acid from a band containing the 4-chloro-2-methyl and 6-chloro-2-methyl α-acids, diethyl ether tended to increase the rate of elution of all acids and to elute the 4:6-dichloro-2-methyl and 4-chloro-2-methyl acids as one band, 6-chloro-2-methylphenoxypropionic acid being the isolated component in this instance.

The effect of changing the pH of the static phase is such that an increase renders the acids more soluble, and, although the degree of separation is better, the elution peaks are less sharp and the time of elution is longer.

It was hoped that, by using a suitable solvent mixture and static phase, complete separation of the four acids might be possible, but it was soon found that the pH required to achieve this would be so high as to make the procedure lengthy and impracticable.

Ultra-violet absorption measurements of solutions of each acid showed that, in the wavelength region around 287 mμ, the 4-chloro-2-methyl acid absorbed strongly and 6-chloro-2-methylphenoxypropionic acid had negligible absorption.

It was then decided to separate the four components into three main bands containing (*i*) 4:6-dichloro-2-methylphenoxypropionic acid, (*ii*) 4-chloro-2-methyl- and 6-chloro-2-methylphenoxypropionic acids, and (*iii*) 2-methylphenoxypropionic acid, and to measure the ultra-violet absorption of the second band at 287 mμ. From our experience, all light absorption at this wavelength could be attributed to the 4-chloro-2-methyl acid. The most rapid way of effecting the chromatographic separation was found to necessitate a

static phase at pH 7·3 and a moving phase consisting of a mixture of equal volumes of diethyl ether and chloroform. A typical elution curve for this system is shown in Fig. 1, the columns having been calibrated by applying a synthetic mixture and then titrating the eluate with 0·01 *N* sodium hydroxide.

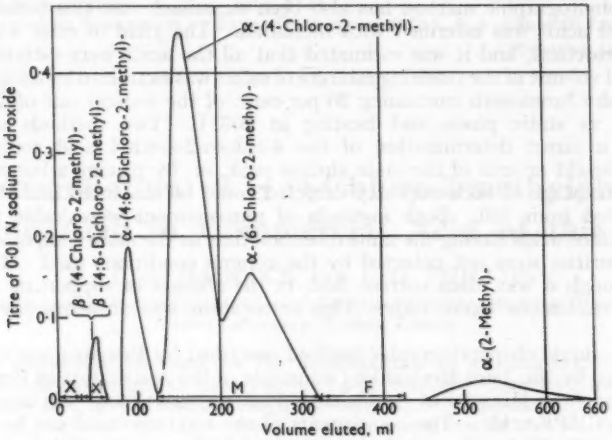

Fig. 1. Elution of phenoxypropionic acids. The static phase was 12·5 ml of 0·25 *M* phosphate buffer solution (pH 7·3) on 25 g of Hyflo Super-Cel, the moving phase was an equilibrated mixture of ether and chloroform (1 + 1), the flow rate was 2 ml per minute (3 lb per sq. inch) and the column loading was 8 mg of acids

The determination involves collecting the appropriate volumes of eluate shown in Fig. 1 as X, X′, M and F. Solutions X and X′ always consist of the first and second 30-ml portions (0 to 30 ml and 30 to 60 ml), respectively, but the range collected for solutions M and F depends on the calibration curve. The volume collected for solution M depends on how sharply the acids are being eluted; normally, 200 ml suffices, and, even if the volume in which the main band is eluted is smaller, it is convenient to collect it in a 200-ml fraction. Solution F always has a volume of 100 ml and is collected immediately after elution of the main band and collection of solution M.

The background optical density of the eluate (uncontaminated by acids) was fairly constant, and any difference in optical density (x) between solutions X and F is assumed to have arisen from a shift in value, the shift changing linearly with the volume eluted.

The optical density of solution M can be measured against solution F, and, after correction for background optical density, the amount of active acid can be read directly from a calibration graph.

The reliability of the method depends on the accuracy of this reading: the reading is usually large (about 1), so that it is not easily determined with any certainty.

In order to increase the accuracy of the method, the optical density of solution M is not measured directly against solution F, but against a solution, G, which consists of pure 4-chloro-2-methylphenoxypropionic acid in solution F. Solution G has such a concentration that, neglecting all changes in background optical density, it would correspond exactly to solution M if the latter were 200 ml in volume and the acid put on the column had been 8 mg of pure 4-chloro-2-methylphenoxypropionic acid.

The relatively low optical density (h) can be accurately measured with a Unicam SP500 spectrophotometer.

The optical density of solution G against solution F (z) is large, but the accuracy of measurement is adequate to permit its use in equation (1).

If, then, it is valid to assume that any background optical-density drift is linear with respect to volume eluted, it can be shown by considering the geometry of the problem that the percentage of α-(4-chloro-2-methylphenoxy)propionic acid is given by—

$$\frac{z+\frac{x}{3}-h}{5z} \times \frac{\text{Volume of solution M, ml}}{\text{Weight of sample, g}} \quad .. \quad .. \quad .. \quad (1)$$

(where the weight of sample is as described under "Method," *i.e.*, fifty times greater than that applied to the column).

All these values, except z, can be reliably determined. Since z appears in both numerator and denominator, however, and as x is always small and h is usually small by comparison, any small inaccuracy is not significant.

INTERFERENCE FROM OTHER CONSTITUENTS IN CMPP FORMULATIONS—

Other constituents known to be present in a few CMPP formulations are certain substituted β-phenoxypropionic acids. Some of these acids have been examined, and their ultra-violet absorption spectra were found to be similar to those of the α-analogues.

The β-(4-chloro-2-methyl)- and β-(4 : 6-dichloro-2-methylphenoxy)propionic acids absorb significantly at 287 mμ and have been examined chromatographically under the conditions described previously. They have been found not to interfere with the determination, as they are eluted from the column before any α-acids appear in the eluate.

METHOD

The chromatographic separation is similar to that used in the examination of MCPA.[1] The four acids, 4 : 6-dichloro-2-methyl-, 4-chloro-2-methyl-, 6-chloro-2-methyl- and 2-methylphenoxypropionic acids, are separated by partition chromatography, kieselguhr and phosphate buffer being used as static phase and diethyl ether - chloroform mixture as moving phase; separation of the 2-methyl and 4 : 6-dichloro-2-methyl acids is complete, but the 4-chloro-2-methyl and 6-chloro-2-methyl acids are only partly separated. The column is standardised with pure acids, and the 4-chloro-2-methyl acid is then determined in a sample by collecting the fraction of the eluate containing the mixed 4-chloro-2-methyl and 6-chloro-2-methyl acids and measuring its ultra-violet absorption at 287 mμ, at which wavelength the 4-chloro-2-methyl acid absorbs strongly, but the 6-chloro-2-methyl acid has negligible absorption. The packing of the column does not appear to be so critical as in the method for MCPA.

APPARATUS—

Chromatographic tube—A glass tube 50 cm in length having an internal diameter of between 1·55 and 1·65 cm. The tube is constricted at its lower end and has a B19 socket at its upper end, into which a tap funnel fits.

Packer—A stainless-steel disc of diameter 1 mm less than the internal diameter of the chromatographic tube. The disc has six holes $\frac{1}{16}$-inch in diameter and a centrally located rod ($\frac{1}{8}$-inch in diameter and about 60 cm in length).

Burette—A 5-ml microburette fitted with a soda-lime - asbestos guard-tube. The burette should be calibrated in 0·01 or 0·02-ml divisions and should be capable of delivering a 0·02-ml drop.

Pipette—To deliver a volume of 1 ml between two graduations.

Unicam SP500 *spectrophotometer.*

REAGENTS—

All materials must be of recognised analytical-reagent grade.

Kieselguhr—Hyflo Super-Cel (obtained from Johns-Manville Ltd., Artillery Row, London, S.W.1).

Buffer solution A, pH 7·3—Mix 165 ml of 0·25 *M* disodium hydrogen orthophosphate and 35 ml of 0·25 *M* sodium dihydrogen orthophosphate. Check the concentration of the disodium hydrogen orthophosphate solution by titration against 0·25 *N* hydrochloric acid

(use bromocresol green as indicator and the 0·25 *M* sodium dihydrogen orthophosphate as a standard for the end-point). Check the concentration of the sodium dihydrogen orthophosphate solution by titration against 0·25 *N* sodium hydroxide (use thymol blue as indicator and the 0·25 *M* disodium hydrogen orthophosphate as a standard for the end-point).

Alternatively, dissolve 5·857 g of anhydrous disodium hydrogen orthophosphate and 1·366 g of hydrated sodium dihydrogen orthophosphate, $NaH_2PO_4.2H_2O$, in distilled water, and dilute the solution to 200 ml. Check the purity of the sodium dihydrogen orthophosphate by titration against *N* sodium hydroxide with thymol blue as indicator (1 ml of *N* sodium hydroxide ≡ 0·156 g of hydrated sodium dihydrogen orthophosphate), and adjust the weight used if necessary. Check the purity of the disodium hydrogen orthophosphate by titration against *N* hydrochloric acid with bromocresol green as indicator (1 ml of *N* hydrochloric acid ≡ 0·142 g of disodium hydrogen orthophosphate).

Diethyl ether - chloroform mixture—Mix equal volumes of diethyl ether and chloroform, cool to room temperature, and keep the mixture protected from strong light. This is solution B.

Solvent mixture—Equilibrate solutions A and B at room temperature by shaking 1 litre of solution B and 50 ml of solution A in a separating funnel. Cool the mixture to room temperature, and filter the lower layer through cotton-wool to remove traces of suspended aqueous layer. This is solution C.

Sodium hydroxide solution (free from carbon dioxide), 0·01 N—Prepare this solution in the way described by Davies and Nancollas,[2] and store it in an aspirator bottle protected from atmospheric carbon dioxide. Alternatively, 0·01 *N* barium hydroxide, which must also be protected from atmospheric carbon dioxide, can be used.

Solution D—Dissolve 0·1 g of 4:6-dichloro-2-methylphenoxypropionic acid, 0·5 g of 4-chloro-2-methylphenoxypropionic acid and 0·2 g each of 6-chloro-2-methylphenoxypropionic acid and 2-methylphenoxypropionic acid in 100 ml of solution B.

Solution E—Dissolve exactly 0·2 g of 4-chloro-2-methylphenoxypropionic acid in 100 ml of diethyl ether.

Ethanol, absolute.

Bromothymol blue indicator solution.

PREPARATION OF COLUMN—

Place 25 g of Hyflo Super-Cel in a mortar, and add, with careful mixing, 12·5 ml of buffer solution A. Triturate gently for several minutes. Add about 250 ml of equilibrated solvent mixture C, and again triturate gently for 3 to 4 minutes.

Close the lower end of the chromatographic tube (which should be cut square and not obliquely) by means of a cork, and hold it loosely in two clamps. For convenience, place the lower clamp below the constriction and the upper clamp near the top of the column. The tube can now be rotated easily, but will remain firm under vertical pressure.

Pack a firm wad of cotton-wool that has been extracted with diethyl ether to form a base for the column, and add solvent to fill about two-thirds of the tube. Maintain this depth of liquid above the packed solid throughout the packing operation. Add a little of the slurried Hyflo Super-Cel, and vigorously agitate with the packer disc to remove any entrained air. Pack the first 0·5 cm of the tube firmly, so as to form a sound base for the column.

Pack the remainder of the column at a sufficient pressure, as found by experience. Hold the packer disc about 3 to 4 cm from the surface of the packed material, and slowly push down so as to impact 1 to 2 mm of material. Press firmly down, and then, with short rapid strokes, consolidate the edges of the column by holding the disc against the side of the tube and rotating both tube and packer. The rapid motion of the packer disc during this operation prevents the impacting of the new material. Repeat this process until all the Hyflo Super-Cel has been packed in 1 to 2-mm sections.

The consolidation of the edges of the column after each section has been packed is most important. Large amounts of thick slurry must not be pushed down and impacted quickly, as this leads to entrainment of air in the column. Build up the column slowly and uniformly, the entire operation should take 1 hour; each addition of slurry, with subsequent packing, results in a uniformly packed column. The length of the packed column should be 26 to 34 cm. If the length of the packing is not within this range, then either the trituration or the packing has not been done correctly. Longer and more vigorous trituration or harder packing, or both, will produce a shorter column.

STANDARDISATION OF COLUMN—

Assemble the apparatus as shown in Fig. 2. There will be about 20 to 30 ml of solvent above the packed Hyflo Super-Cel. This may be used to ascertain the pressure of nitrogen (applied through A) required to give the necessary flow rate of 2 to 2·5 ml per minute. The pressure required should be 3 to 5 lb per sq. inch (as shown on the gas-cylinder gauge). If the pressure required is much greater than this, the column has probably been packed too tightly.

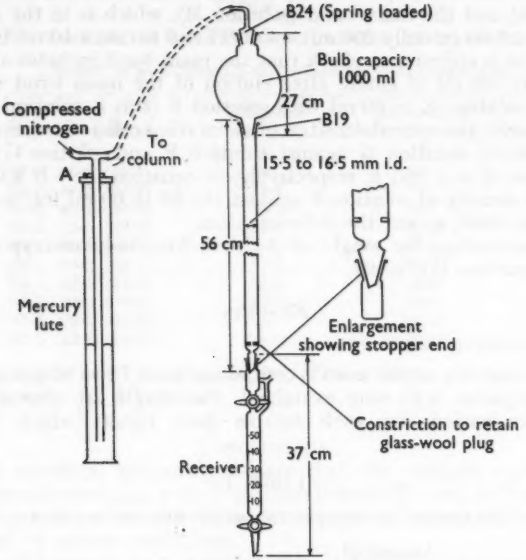

Fig. 2. Column assembly

Allow the level of the liquid above the column to fall until only an extremely thin layer of liquid remains above the Hyflo Super-Cel. Remove the separating funnel, and place 1 ml of solution D on the surface by means of a 1-ml pipette. Force this liquid through the column by pressure of nitrogen until the 1-ml portion of solution D has just been absorbed. Follow the same procedure with two successive 1-ml portions of solution C. Finally, replace the separating funnel containing solution C, and pass about 1 litre of this solution through the column. Collect successive 10-ml portions of the eluate, and remove the solvent by evaporation on a water bath in the presence of 3 to 4 ml of water. When all the diethyl ether - chloroform mixture has been removed, titrate each fraction with 0·01 *N* alkali. Pass a rapid stream of carbon dioxide-free air or nitrogen through the liquid during titration. The stream of gas should pass through the liquid for at least 2 minutes before the titration is begun in order to remove any dissolved carbon dioxide. From the results of these titrations, standardise the column by plotting volume eluted against titration of last 10-ml fraction (see Fig. 1). If the troughs between the peaks are not well defined, the column has not been properly packed and another must be prepared.

Non-uniform packing of the Hyflo Super-Cel will cause poor separation of the acids, and variation of the concentrations of buffer salts from those required will affect the resolution and positions of the peaks.

Provided that the standardisation curve is satisfactory, the column may be run "blind" at the same flow rate. It is advisable to check the column after four determinations and after storage. Take care to prevent the column from becoming dry, and, if a column is set aside for longer than 2 days, flush it with 50 ml of freshly equilibrated solvent before use.

PROCEDURE—

Prepare enough solution C to last the whole determination, and mix thoroughly. (Equilibrate the solvent mixture and the buffer solution immediately before use.) Pass

50 ml of solution C through the column, and allow the level of the liquid to fall to the level of the Hyflo Super-Cel.

Weigh accurately about 0·4 g of acids extracted from CMPP, and make up to 50 ml with diethyl ether. By pipette, carefully place 1 ml of this solution on the column, and wash it on with two successive 1-ml portions of solution C. Elute with the freshly prepared solution C (pass about 1 litre to ensure that the unchlorinated 2-methylphenoxypropionic acid is removed from the column). Collect the first 30 ml of eluate, the next 30 ml (*i.e.*, the 30 to 60-ml fraction) and the main band (solution M), which is in the appropriate volume determined by calibration (usually 200 ml). Collect and titrate a 10-ml fraction immediately before the main band is eluted as a check that the main band includes all the 4-chloro acid.

Collect the next 100 ml of eluate after elution of the main band (this is solution F).

Dilute 1 ml of solution E to 50 ml with solution F (this is solution G).

At 287 mμ, measure the optical densities (in 4-cm silica cells) of solution F against the first 30-ml portion of eluate, solution G against solution F and solution G against solution M. This gives the values of x, z and h, respectively, in equation (1). If x is greater than 0·05, measure the optical density of solution F against the 30 to 60-ml fraction of eluate, and, if x is still greater than 0·05, repeat the determination.

Calculate the percentage by weight of 4-chloro-2-methylphenoxypropionic acid in the sample by using equation (1), p. 97.

Results

Application to synthetic mixtures—

Five synthetic mixtures of the α-acids containing from 75 to 95 per cent. of α-(4-chloro-2-methylphenoxy)propionic acid were examined; the results are shown in Table I.

Replicate determinations on each sample gave results which were satisfactorily reproducible.

Table I

Analysis of synthetic mixtures of α-acids

Mixture	Amount of 4-chloro-2-methylphenoxypropionic acid present, %	Amount of acid found, %	Average, %
A*	75	75·4, 75·7, 76·1, 77·1	76·1
B†	80	81, 80·1, 78·0	79·7
C‡	85	87·1, 85·3, 83·2, 84·6	85·1
D§	90	91·0, 89·8, 89·8, 90·0	90·2
E‖	95	96·3, 95·9, 95·0, 94·3	95·4

* Mixture A also contained 10 per cent. each of 4:6-dichloro-2-methylphenoxypropionic acid and 6-chloro-2-methylphenoxypropionic acid and 5 per cent. of 2-methylphenoxypropionic acid.

† Mixture B also contained 5 per cent. each of 6-chloro-2-methylphenoxypropionic acid and 2-methylphenoxypropionic acid and 10 per cent. of 4:6-dichloro-2-methylphenoxypropionic acid.

‡ Mixture C also contained 5 per cent. each of 4:6-dichloro-2-methylphenoxypropionic acid, 6-chloro-2-methylphenoxypropionic acid and 2-methylphenoxypropionic acid.

§ Mixture D also contained 5 per cent. each of 4:6-dichloro-2-methylphenoxypropionic acid and 6-chloro-2-methylphenoxypropionic acid.

‖ Mixture E also contained 2·5 per cent. each of 4:6-dichloro-2-methylphenoxypropionic acid and 6-chloro-2-methylphenoxypropionic acid.

These results were used to calculate the standard deviation of a single determination. From this value, the limits of 95 per cent. confidence, *i.e.*, the range in which nineteen out of twenty results of single determinations would be expected to lie, were calculated; the limits were ±2·8 per cent.

Comparison with other methods—

In addition to partition chromatography, commercial formulations have also been examined by gas chromatography of the esters, infra-red analysis and a differential refractometric method. Ten samples were examined, and the results by different methods are shown in Table II.

These results show that, in general, agreement between the proposed and the infra-red methods was good. In all but one instance, results obtained by gas chromatography of the

esters were higher; it must be remembered, however, that chromatographic methods tend to give a maximum value, since the presence of any unknown material having the same retention time as the main band will lead to a positive error.

The differential refractometric method has been shown, in certain instances, to be considerably affected by small amounts of tarry material occurring in formulations, and hence it is not always reliable for the analysis of CMPP.

TABLE II

ANALYSIS OF COMMERCIAL CMPP FORMULATIONS BY VARIOUS METHODS

Sample	Amount of α-(4-chloro-2-methylphenoxy)propionic acid found by— proposed method, %	infra-red analysis, %	differential refractometry, %	gas chromatography, %
A	85·0, 85·2 (85·1)	86	84	90
B	80·1, 80·3 (80·2)	83·7	77	87·6
C	87·0, 86·1 (86·5)	86·7	80	90
D	82·5, 82·5 (82·5)	81·2	85·6	83·4
E	81·8, 82·8 (82·3)	—	79·5	90
F	96·1, 96·2 (96·1)	97·1	93·2	—
G	90·7, 92·5 (91·6)	91·6	85·6	93·9
H	94·3, 94·6 (94·4)	93·7	90·9	93·5
I	93·3, 92·2 (92·8)	—	92·6	95·4
J	96·5, 95·8 (96·2)	—	94·0	97·0

These results were also used to calculate the limits of 95 per cent. confidence, which, for commercial samples, were ±1·5 per cent.

CONCLUSIONS

The results for synthetic mixtures indicate that, for samples containing α-(4-chloro-2-methyl)-, α-(6-chloro-2-methyl)-, α-(4:6-dichloro-2-methyl)- and α-(2-methylphenoxy)propionic acids, the proposed method is reliable for samples of mixed acids containing at least 75 per cent. or more of active constituent.

The procedure, although it may seem lengthy, is simple to carry out. Interference by an unknown substance would occur only if that substance had the same retention time (under the conditions used) as α-(4-chloro-2-methylphenoxy)propionic acid and an appreciable ultra-violet absorption at 287 mμ. This possibility is remote.

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The Determination of Phenkaptone Residues in Fruit and Vegetables

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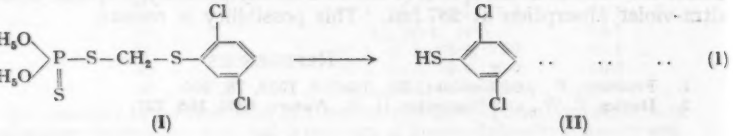
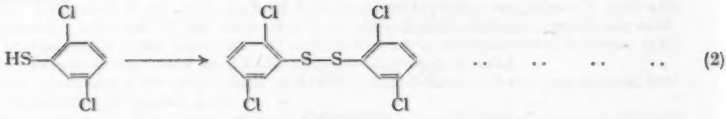
A procedure is described for the determination of S-(2:5-dichlorophenylthiomethyl) OO-diethyl phosphorothiolothionate residues in fruit and vegetables. Bromination of the 2:5-dichlorothiophenol formed by hydrolysis of the pesticide is carried out in acetic acid. The resulting 2:5-dichlorophenylsulphonylbromide reacts with potassium cyanide to form cyanogen bromide, which is determined colorimetrically by Aldridge's method. Plant waxes in the extract are removed with acetonitrile. The procedure is relatively specific for phenkaptone. Other pesticides containing thiophosphoric acid esters interfere slightly; carbon disulphide and hydrogen sulphide do not interfere. The sensitivity of the method is about 5 μg in a 100-g sample, *i.e.*, 0·05 p.p.m.

The organo-phosphorus compound S-(2:5-dichlorophenylthiomethyl) OO-diethyl phosphorothiolothionate (phenkaptone) is a persistent acaricide having ovicide properties. Its residues in plant material may be determined in the usual way for organic phosphates, namely, by distilling the extracted residue *in vacuo*, converting to phosphate and determining the latter colorimetrically. This method is not specific, of course, and gives recoveries of between 60 and 70 per cent. In an attempt to establish a more reliable method, we studied a reaction described by Saville.[1] He observed that thiolgenic compounds, including aliphatic thiols, when treated with bromine water, are converted to the corresponding alkylsulphonyl bromides, which react with potassium cyanide to form potassium alkylsulphinates and cyanogen bromide. The latter can readily be determined colorimetrically by Aldridge's method,[2] which is based on the König reaction with pyridine and benzidine; this results in the formation of the red dianil of glutacondialdehyde.

On alkaline hydrolysis, phenkaptone (**I**) could be expected to yield 2:5-dichlorothiophenol (**II**, m.p. 27° to 28° C),[3] and this, on oxidation, to yield bis-(2:5-dichlorophenyl)-disulphide (**III**, m.p. 81° C),[4] as shown by the following reactions—



(I) (II) .. .. .. (1)



(III) .. .. (2)

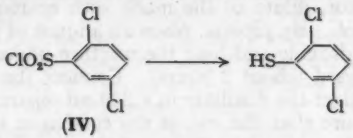
If Saville's reaction applied to aromatic thiols, 2:5-dichlorothiophenol could be determined by Aldridge's method.

Experimental

Saville's reaction is not necessarily restricted to aliphatic thiols. Thiophenol gave the reaction, but the colour formed in aqueous solution was only faint, the optical density (1-cm cell) of a solution of 50 μg of thiophenol in 25 ml of water being 0·107 at 535 mμ. When the reaction was carried out in glacial acetic acid, however, the optical density was 0·448.

Phenkaptone was heated with aqueous sodium hydroxide, the mixture was acidified and steam-distilled, and the distillate was extracted with methylene chloride. The extract yielded a substance with a mercaptan-like smell, which, after recrystallisation from glacial acetic acid, melted at 24° C. Recrystallisation from an ethanol - water mixture gave a

product melting at 81° C. To confirm that these were (II) and (III), respectively, 2:5-dichlorophenylsulphonylchloride (IV) was reduced (see reaction 3) to the thiophenol, m.p. 24° C, which was easily oxidised to the disulphide, m.p. 81° C.



(IV) .. .. (3)

Both 2:5-dichlorothiophenol and the corresponding disulphide gave Saville's reaction; optical-density values were again higher when the bromination was carried out in glacial acetic acid. In water and glacial acetic acid, respectively, the optical densities of solutions containing 40 μg of 2:5-dichlorothiophenol per 25 ml were 0·235 and 0·492.

As 2:5-dichlorothiophenol is highly volatile, the procedure must be carefully controlled if results are to be reproducible. When the phenkaptone has reacted with aqueous sodium hydroxide, the distillation flask containing the mixture is connected to a steam generator, and air is removed from the apparatus before sulphuric acid is added. The rate of steam-distillation must be carefully controlled and the tip of the condenser must be kept beneath the surface of a small amount of methylene chloride in the receiver.

Mercuric acetate in acetic acid is added to the methylene chloride extract to prevent evaporation of 2:5-dichlorothiophenol during removal of the solvent.

The presence of plant waxes in fruit and vegetable extracts appears to interfere with Saville's reaction, as it was found that, when the bromination was carried out in a turbid solution, the subsequent optical-density values were low.

Attempts to separate waxes from the phenkaptone fraction by chromatography were unsuccessful, owing to losses of phenkaptone. However, results were satisfactory when the extracted plant residue was dissolved in warm acetonitrile and the cooled solution was filtered. (The acetonitrile is hydrolysed by an excess of sodium hydroxide.) When 2:5-dichlorothiophenol and bromine react in glacial acetic acid, the small amounts of plant waxes remaining after treatment with acetonitrile do not interfere with the reaction and precipitation is inhibited.

Method

Reagents—

Light petroleum—The fraction boiling over the range 20° to 40° C.

Glacial acetic acid.

Mercuric acetate solution—Prepare a 5 per cent. w/v solution of mercuric acetate in glacial acetic acid.

Acetonitrile.

Sodium hydroxide, 8 N—Prepare from analytical-reagent grade material.

Sulphuric acid, 60 *per cent. w/v.*

Methylene chloride—Wash commercial-grade methylene chloride with concentrated sulphuric acid and with 4 *N* sodium hydroxide. Thoroughly wash with water, and distil.

Bromine solution—Dilute 1 ml of a 4 per cent. w/v solution of bromine in glacial acetic acid to 5 ml with glacial acetic acid. Prepare this solution daily. (In a refrigerator, the 4 per cent. solution can be kept for 1 week.)

Phenol solution—Dissolve approximately 8 g of phenol in 100 ml of 5 per cent. potassium bromide solution.

Potassium cyanide solution, aqueous, 10 *per cent. w/v.*

Aldridge's reagent solution—Add 10 ml of concentrated hydrochloric acid to a mixture of 30 ml of distilled pyridine and 70 ml of water; this is solution *A*. Prepare a 5 per cent. w/v solution of analytical-reagent grade benzidine hydrochloride in 2 per cent. hydrochloric acid; this is solution *B*. Shortly before use, mix 3 ml of solution *A* with 0·6 ml of solution *B*.

Ethanol, 96 *per cent.*

Sodium sulphate, anhydrous.

Procedure—

Place 500 to 1000 g of the sample in an Erlenmeyer flask, and cover with a measured volume of light petroleum. Set the flask aside overnight, and then decant and filter the liquid.

Place an aliquot (about 100 ml) of the solution in a Kuderna - Danish apparatus and distil the solvent. Remove the last traces of solvent by means of a current of air. Dissolve the residue in hot acetonitrile and transfer the solution quantitatively to a 10-ml calibrated flask. Allow to cool in a refrigerator, dilute to the mark with acetonitrile and filter the solution through a plug of cotton-wool. By pipette, place an aliquot of the filtrate in a 150-ml flask, add 35 ml of 8 *N* sodium hydroxide and heat the mixture under reflux until the acetonitrile has been completely hydrolysed (about 2 hours). Connect the flask to a steam-distillation apparatus and arrange to collect the distillate in a 250-ml separating funnel containing 10 ml of methylene chloride. Ensure that the end of the condenser is beneath the surface of the methylene chloride. Clear the apparatus of air and then carefully add 25 ml of 60 per cent. sulphuric acid, by means of a dropping funnel, to the boiling solution. Continue distillation until 150 ml of distillate have been collected (30 to 40 minutes). Shake for 1 minute, allow the layers to separate and filter the methylene chloride layer through a cotton-wool plug covered with 1 g of anhydrous sodium sulphate into a 25-ml calibrated flask containing 5 drops of mercuric acetate solution and a few chips of pumice stone. Repeat the extraction and filtration procedure twice with 10-ml portions of methylene chloride. Gently evaporate on a steam-bath and remove the last traces of methylene chloride by means of a current of air.

By pipette, add 0·5 ml of bromine solution to the residue (take care that the reagent does not touch the neck of the flask). Set aside for 20 minutes in a refrigerator, add 0·2 ml of phenol solution and allow exactly 2 minutes for this to react with excess of bromine. Add 1 drop of potassium cyanide solution (which is sufficient for the formation of the cyanogen bromide) and, after another 2 minutes, 5 ml of Aldridge's reagent solution. Place the flask in a refrigerator for 20 minutes and then dilute to the mark with ethanol. After 15 minutes, measure the optical density of the solution in a 1-cm absorption cell at 535 mμ.

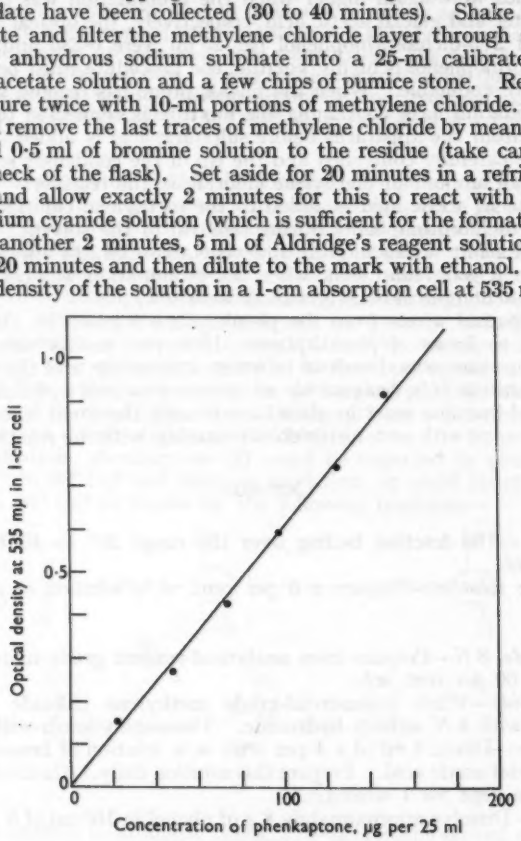


Fig. 1. Calibration graph

PREPARATION OF A CALIBRATION GRAPH—

Place solutions containing between 20 and 200 μg of phenkaptone dissolved in methylene chloride in a series of flasks and, after removal of the solvent, hydrolyse each with sodium hydroxide. Follow the procedure described and then measure the optical densities of the final solutions with a Unicam SP500 spectrophotometer. Construct a graph of optical density against phenkaptone concentration, as shown in Fig. 1. Optical-density values from weighed samples of 2:5-dichlorothiophenol prepared by hydrolysis of phenkaptone and also by reduction of 2:5-dichlorophenylsulphonylchloride were in good agreement with corresponding values on the calibration graph; this shows that the phenkaptone used was of a satisfactory purity.

Results

The percentage recovery with the procedure was determined by analysing extracts of lettuce, French beans and apples to which known amounts of phenkaptone had been added. The results are shown in Table I. The optical-density values for phenkaptone-free extracts of lettuce, French beans and apples were 0·026, 0·015 and 0·025; these values correspond to phenkaptone concentrations of, respectively, 4·2, 1·4 and 4·0 μg per 100 ml of extract.

To determine the specificity of the method, a series of related compounds that might possibly interfere was subjected to the procedure described. Solutions of dimethyl S-(2-ethylthioethyl) phosphorothiolothionate (thiometon), a mixture of diethyl S-(2-ethylthioethyl) phosphorothiolate (demeton-S) and diethyl (2-ethylthioethyl) phosphorothionate (demeton-O), diethyl *p*-nitrophenyl phosphorothionate (parathion) and S-1:2-di(ethoxycarbonyl)ethyl dimethyl phosphorothiolothionate (malathion) were analysed.

Extracts to which 100 μg of these substances had been added gave slightly coloured solutions, which suggests that residues of these chemicals interfere. When the hydrolysis was carried out at room temperature instead of at boiling-point, optical-density values for thiometon were much higher. In addition, as shown by Groves,[5] demeton-S is hydrolysed by cold 2 *N* sodium hydroxide - methanol solution to a thiol, which reacts with bromine to give ethylsulphinylethyl sulphonylbromide. Methods for determining residues of thiometon and demeton-S may possibly be based on this reaction, and this is being studied. Neither carbon disulphide nor hydrogen sulphide added after hydrolysis with sodium hydroxide showed interference.

Table I

Recovery of phenkaptone from plant extracts

Sample	Amount of phenkaptone added, μg	Amount of phenkaptone found, μg	Recovery, %
Lettuce	100	94	94
	125	121	97
French beans	50	46	92
	100	95	95
	150	150	100
Apple	25	25	100
	50	46	92
	100	96	96
	150	152	101

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The Separation and Determination of Rhodium and Iridium

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An accurate method for the separation and determination of rhodium and iridium in a wide range of ratios has been developed. Rhodium is separated quantitatively as an insoluble bivalent complex with thioacetanilide by reduction with chromous chloride, and the iridium in the filtrate is precipitated as sulphide by thiourea. The precipitates are ignited and finally weighed as metal.

The separation can be used for the determination of small amounts of iridium in rhodium.

In the analysis of the platinum metals, the separation of rhodium from iridium is one of the most difficult to carry out accurately, particularly when both are present in approximately equal amounts. Numerous methods have been proposed, and these can be summarised as follows—

(*a*) Selective reduction of rhodium to metal,[1 to 5]
(*b*) ion-exchange and chromatographic methods,[6 to 12] and
(*c*) electrolytic and other methods.[13,14,15]

A number of these procedures have distinct shortcomings in accuracy, and almost all the others have difficulties in that they either necessitate double precipitation of one or both elements or are only applicable to limited ratios and amounts of the two metals.

A few organic reagents have been used in the determination of rhodium,[16,17,18,19] and at least two of these[16,18] formed complexes in which the element was present in the bivalent state. (These complexes were prepared by boiling with the reagent.) The presence of iridium invariably influenced the accuracy.

Pollard[20] has shown that rhodium can be precipitated in the bivalent state by adding titanous sulphate to a rhodium sulphate solution containing 2-mercaptobenzothiazole and that the iridium can be subsequently determined in the filtrate. Unfortunately, the voluminous rhodium precipitate restricted the application of the method to micro work, and the use of titanous sulphate, which is easily hydrolysed, made the gravimetric determination of rhodium uncertain.

Nevertheless, the principle of precipitating a bivalent rhodium compound appeared to offer distinct possibilities for separating the two metals accurately, if more suitable reagents could be found.

Experimental

As alternative reducing agents to titanous sulphate, chromous and hypovanadous salts were found to be satisfactory for the separation in which 2-mercaptobenzothiazole was used and had the advantage of not hydrolysing under the conditions of the method. A number of organic thio compounds were investigated as possible reagents for rhodium, and the majority of those tested gave precipitates on addition of chromous chloride or hypovanadous chloride at room temperature. Reagents previously used in the determination of rhodium, thiobarbituric acid,[16] mercaptobenzoxazole[17] and 2-mercapto-4:5-dimethylthiazole,[18] all reacted in the cold when the reducing agent was added. The complexes formed by the first two reagents were insoluble, but that formed by the third was soluble and coloured. (Extraction of this complex with chloroform from a sulphate solution containing hydrochloric acid gave a separation of rhodium from iridium.) A number of thioureas, *e.g.*, diphenylthiourea, α- and β-naphthylthioureas, *sym.*-di-*o*-tolylthiourea, thiourea dioxide, benzyl*iso*thiourea hydrochloride, phenylthiohydantoic acid and S-methyl*iso*thiourea sulphate, also formed insoluble compounds under these conditions. Thioacetic acid, thioformaldehyde, thioacetanilide, thiophenol, toluene-*p*-thiol, *o*-mercaptobenzoic acid, dithio-oxamide, sodium diethyldithiocarbamate, dithizone, dimercaptothiodiazole, thionalide, thioresorcin and thiodiphenylamine also formed precipitates.

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Thioacetanilide, $C_6H_5{\cdot}NH{\cdot}CS{\cdot}CH_3$, appeared to have a distinct advantage in that the flocculent dirty-yellow precipitate became more granular as the concentration of rhodium increased; a precipitate containing 100 or 150 mg of the metal could be easily separated on an 11-cm filter-paper.

PRECIPITATION OF RHODIUM WITH THIOACETANILIDE—

Thioacetanilide and freshly prepared chromous chloride solution were added to solutions containing different amounts of rhodium, the solutions being stirred during the additions. The precipitates were stirred occasionally, and, after about 2 hours, were removed by filtration, washed well and ignited slowly. The precipitates were then reduced in a stream of hydrogen, treated with hydrofluoric acid in platinum dishes and evaporated to dryness on a hot-plate to remove silica. After being moistened with diluted hydrochloric acid, the residues were separated by filtration, washed, ignited, reduced in hydrogen and weighed.

Tests on these precipitates indicated slight contamination by chromium; the precipitates were therefore heated in chlorine at 650° to 700° C and then leached with dilute aqua regia. When the insoluble rhodium chloride was removed by filtration, ignited, reduced and weighed, the results were in close agreement with the amounts of rhodium taken. This procedure was adopted in subsequent work.

The results of the experiments with thioacetanilide were as follows—

Amount of rhodium present, mg	0·91	22·6	45·8	91·6
Amount of rhodium found without heating in chlorine, mg ..	0·92	22·65	46·05	92·05
Amount of rhodium found after heating in chlorine, mg ..	0·90	22·55	45·8	91·8

COMPOSITION OF THE RHODIUM COMPLEX—

The complex formed with thioacetanilide was precipitated by chromous chloride from a solution approximately 0·2 *N* in hydrochloric acid. After being removed by filtration, the precipitate was dissolved in glacial acetic acid and the solution was filtered. The complex was re-precipitated by adding diluted hydrochloric acid (1 + 9), removed by filtration, washed well with water and dried to constant weight over silica gel.

Analysis of this complex showed the presence of 13·65 per cent. of rhodium and 9·35 per cent. of chlorine.

The hypothetical bivalent co-ordination compound, $Rh(C_6H_5{\cdot}NH{\cdot}CS{\cdot}CH_3)_4Cl_2$, would contain 13·22 per cent. of rhodium and 9·11 per cent. of chlorine and would conform in structure to the bivalent co-ordination compounds prepared by Dwyer and Nyholm.[21]

SEPARATION OF RHODIUM FROM IRIDIUM—

Attempts to separate rhodium from iridium by means of thioacetanilide were first carried out in a chloride solution, but co-precipitation of iridium was considerable. Further separations were made in sulphate solutions prepared by evaporating the chloride solutions with nitric acid and sulphuric acid containing lithium sulphate[20] until fumes were evolved and then diluting with water. Satisfactory separations were achieved. For example, from a solution containing 45·8 mg of rhodium and 50·95 mg of iridium, 45·9 mg of rhodium were recovered. On analysis, this precipitate was found to contain 0·06 mg of iridium.

RECOVERY OF IRIDIUM—

After removal of the rhodium precipitate, the iridium in the filtrate was separated quantitatively from the chromium by a modification (communicated privately by Dr. W. B. Pollard) of Pollard's thiourea method[20] and was determined gravimetrically. It was found that this procedure was satisfactory for separating iridium provided that there was no prolonged heating at the higher temperatures, otherwise there was a tendency for an insoluble chromium compound to separate.

When the amount of iridium was less than about 0·4 mg, it was best determined volumetrically as described by Pollard.[20] When a 100-mg sample of rhodium was used, it was possible, by this means, to determine iridium contents as low as 0·005 per cent. in refined rhodium sponge.

SEPARATION OF SMALL AMOUNTS OF RHODIUM FROM IRIDIUM—

From a solution approximately 2 *N* in hydrochloric acid, the rhodium complex was readily extracted by chloroform, *n*-butyl alcohol or *iso*amyl alcohol. Attempts to utilise such an extraction for separating small amounts of rhodium from iridium were unsuccessful.

From chloride solutions, some iridium accompanied the rhodium into the solvent, and from sulphate solutions, even when hydrochloric acid was added, extraction of rhodium was not complete.

METHOD

REAGENTS—

Sulphuric acid - lithium sulphate solution—Add 266 g of lithium sulphate to 1 litre of concentrated sulphuric acid, and warm until dissolution is complete.

Thioacetanilide solution—Dissolve 2 g of thioacetanilide in 100 ml of glacial acetic acid, and filter the solution.

Chromous chloride solution, approximately 1·0 N.

Hydroquinone solution—Dissolve 0·1424 g of hydroquinone in 500 ml of dilute sulphuric acid (1 + 99).

3:3′-*Dichlorobenzidine indicator solution*—Warm 0·1 g of 3:3′-dichlorobenzidine with 10 ml of diluted sulphuric acid (1 + 2), and dilute the solution to 100 ml.

PROCEDURE FOR CHLORIDE SOLUTIONS CONTAINING IRIDIUM AND RHODIUM—

Evaporate the chloride solution with 10 ml of concentrated nitric acid and 10 ml of sulphuric acid - lithium sulphate solution until fumes are evolved. Heat strongly, and add 0·5 ml of perchloric acid to ensure complete dissolution. Allow the reaction to subside, and then cool. Dilute, boil, and cool to room temperature.

Dilute to between 200 and 300 ml with cold distilled water, and add approximately 0·5 ml of thioacetanilide solution for each milligram of rhodium present and then 2 to 5 ml of chromous chloride solution. (During this addition, stir the solution, and take care to avoid atmospheric oxidation of the chromous chloride.) Set aside for 2 to 3 hours, and stir the solution occasionally. Filter the solution through an 11-cm Whatman No. 44 filter-paper, wash the rhodium precipitate thoroughly with dilute hydrochloric acid (1 + 99), and ignite gently. Heat in a stream of hydrogen, cool, treat with hydrofluoric acid in a platinum dish, and evaporate to dryness. Moisten the residue with dilute hydrochloric acid (1 + 9), separate on a 9-cm Whatman No. 44 filter-paper, wash with hot water, ignite, and reduce. Heat to a temperature of 650° to 700° C in a current of chlorine, cool, treat with dilute aqua regia (1 + 4), and filter through a 9-cm Whatman No. 44 filter-paper. Ignite the rhodium precipitate, reduce to metal, and weigh.

Evaporate the filtrate containing the iridium with 25 ml each of concentrated nitric and sulphuric acids. Heat on a hot-plate to 250° C (indicated by a 360° C thermometer in the solution), and, without delay, oxidise by adding a few drops of concentrated nitric acid and then perchloric acid. When the reaction has subsided, add 2 to 4 g of solid thiourea, and remove from the hot-plate when the iridium sulphide has flocculated (after a few seconds). Cool, dilute to 250 ml, and separate the precipitate on an 11-cm Whatman No. 41 filter-paper. If there is no evidence of any iridium sulphide, dilute, boil (as it is precipitated, sulphur collects any small amounts of iridium sulphide), and then filter the solution. Wash the precipitate thoroughly with hot water, and carefully ignite. Treat with hydrofluoric acid in a platinum dish, and evaporate to dryness. Moisten with diluted hydrochloric acid, and wash the residue into a small beaker. Add an equal amount of concentrated hydrochloric acid, and boil. Filter the solution through a 9-cm Whatman No. 44 filter-paper, ignite the precipitate, reduce to metal, and weigh.

When the amount of iridium sulphide is extremely small, return the thiourea precipitate to the beaker, and decompose it with 20 ml of concentrated nitric acid and 2 to 3 ml of sulphuric acid - lithium sulphate solution. Evaporate until fumes are evolved, oxidise any remaining carbon with nitric acid and, finally, perchloric acid. Dilute, and filter the solution. Evaporate the solution, and, after oxidation, determine the iridium by titration with hydroquinone solution in the presence of 3:3′-dichlorobenzidine indicator solution, as described by Pollard.[20]

PROCEDURE FOR DETERMINING IRIDIUM IN REFINED RHODIUM SPONGE—

Fuse 100 mg of the sponge with 10 g of zinc and a small amount of ammonium chloride in a covered porcelain crucible. Remove the zinc by dissolution in dilute sulphuric acid

(1 + 9), filter the solution, dissolve the finely divided rhodium in 10 to 15 ml of sulphuric acid - lithium sulphate solution (destroy the filter-paper with nitric acid), and heat until fumes are evolved.

Dilute, cool, and precipitate the rhodium with thioacetanilide as described previously. Add a little filter-paper pulp, set aside for 2 to 3 hours, and stir occasionally during this period. Filter the solution through a pad of filter-paper pulp, wash the residue, and determine iridium in the filtrate volumetrically after separation with thiourea as described previously.

Results

The procedures were applied to a series of synthetic solutions containing accurately known amounts of rhodium and iridium. The results are shown in Table I, together with the amounts of the other metal found in the precipitates.

Table I

Determination of rhodium and iridium by the proposed method

Amount of rhodium present, mg	Amount of iridium present, mg	Amount of rhodium found, mg	Amount of iridium in rhodium precipitate, mg	Amount of iridium found, mg	Amount of rhodium in iridium precipitate, mg
91·6	96·7	91·85	0·20	96·7	0·16
91·6	50·95	91·55	<0·01	50·95	<0·01
45·8	50·95	45·75	0·05	51·05	0·07
45·8	48·35	45·9	0·15	48·25	0·12
22·6	48·35	22·55	0·06	48·25	0·06
9·16	48·35	9·30	0·07	48·25	0·02
0·92	48·35	0·90	0·01	48·10	0·05
9·16	9·67	9·12	<0·01	9·60	<0·01
0·92	9·67	0·90	<0·01	9·60	<0·01
45·8	0·97	45·8	<0·01	0·96	0·06
45·8	0·48	45·85	<0·01	0·48	0·03

The amounts of iridium present in rhodium sponge were also determined; the results were as follows—

Amount of iridium present, % ..	0·092	0·046	0·018
Amount of iridium found, % ..	0·094	0·044	0·015

Interfering elements

Copper, mercury, silver, gold, palladium, osmium and platinum formed precipitates from chloride or sulphate solutions in dilute acid at room temperature with thioacetanilide alone.

Bismuth, ruthenium, rhenium and tungsten formed precipitates from chloride or sulphate solutions in dilute acid at room temperature with thioacetanilide only after the reducing agent had been added. (Precipitation of rhenium and tungsten was extremely slow.)

Metals forming no precipitate from chloride or sulphate solutions in dilute acid after being set aside for 2 hours at room temperature with thioacetanilide either alone or with chromous chloride were lithium, sodium, potassium, magnesium, calcium, strontium, barium, zinc, cadmium, aluminium, titanium, tin, lead, vanadium, arsenic, antimony, chromium, molybdenum, uranium, manganese, iron, cobalt, nickel and iridium.

Conclusions

The proposed method provides an accurate and relatively simple procedure for the separation and determination of iridium and rhodium.

The work suggests that rhodium forms bivalent complexes with numerous organic thio compounds and possibly with other types of organic reagents.

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A Simple Spectrophotometric Method for Determining Magnesium, Calcium, Strontium, Barium, Cadmium and Zinc with Ethylenediaminetetra-acetic Acid

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A spectrophotometric method for the determination of magnesium, calcium, strontium, barium, cadmium and zinc is described. It is based on the measurement of the optical densities of an ethylenediaminetetra-acetic acid solution in the presence and absence of the metal ion, the optical-density difference being used as a quantitative measure of the amount of metal ion present, provided that the metal ion forms a chelate with ethylenediaminetetra-acetic acid and that the chelate has little absorption at the wavelength used. The method is convenient for the rapid analysis of many samples when high accuracy is not required. An accuracy to within ± 3 per cent. can be expected in the analysis of samples containing between 1 and 5 μM of metal ion.

IN a study of the cation-binding properties of cartilage, it was considered to be desirable to develop an analytical method that would permit the metals of groups IIA (excluding radium) and IIB of the periodic system to be determined by a single procedure.

Sweetser and Bricker[1] have made use of the large absorption of ethylenediaminetetra-acetic acid (EDTA) at wavelengths between 222 and 236 mμ to determine end-points spectrophotometrically in the titrations of magnesium, calcium, zinc and cadmium with EDTA. Since these cations and their chelates with EDTA have little absorption at wavelengths above 222 mμ, the end-point of such a titration is indicated by a sudden increase in optical density.

Such titration methods were considered to be inconvenient when many samples had to be analysed, and, further, modifications were necessary so that the spectrophotometer could accommodate the special titration cells.

It was thought that, if the optical densities of an EDTA solution in the presence and absence of a metal ion were known, the optical-density difference could be used as a quantitative measure of the amount of metal ion present, provided that the metal ion forms a chelate with EDTA and that the chelate has little absorption at the wavelength used. A method based on this procedure has been developed.

Experimental

Beryllium, magnesium, calcium, strontium, barium, zinc, cadmium^{II} and mercury^{II} were studied.

In order to test the validity of the suggested method, the absorption spectra at pH 10 of solutions of the metal ions (provided that no precipitation occurred), EDTA and equimolar mixtures of metal ion and EDTA were measured. These measurements were made in the ultra-violet region at between 210 and 300 mμ. The absorption spectra of EDTA and the chelates of magnesium, calcium, strontium, barium, zinc and cadmium with EDTA were similar to those observed by Sweetser and Bricker.[1] The absorption spectra of EDTA, calcium ions *plus* EDTA, beryllium ions *plus* EDTA and mercuric ions *plus* EDTA are shown in Fig. 1.

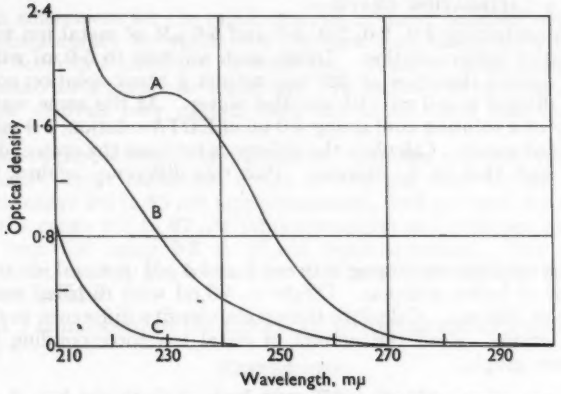

Fig. 1. Absorption spectra: curve A, 0·001 *M* EDTA *plus* 0·001 *M* solution of mercuric ions; curve B, 0·001 *M* EDTA and 0·001 *M* EDTA *plus* 0·001 *M* solution of beryllium^{2+} ions; curve C, 0·001 *M* EDTA *plus* 0·001 *M* solution of calcium^{2+} ions

The curve for calcium ions *plus* EDTA showed little absorption at wavelengths longer than 220 mμ. The spectra of mixtures of EDTA with magnesium, strontium, barium, zinc and cadmium ions were similar to that of a mixture of EDTA and calcium ions.

The curve for beryllium ions *plus* EDTA did not differ from that of EDTA itself, *i.e.*, no chelate compound was formed.

The mercury^{II} - EDTA chelate absorbed strongly at wavelengths between 210 and 260 mμ.

From these results, it appeared that the method would be suitable for determining magnesium, calcium, strontium, barium, zinc and cadmium, but not for determining beryllium and mercury^{II}. At 225 mμ, the optical density of 0·001 *M* EDTA was approximately 1·0, which was considered to be suitable for the determination.

Method

All optical-density measurements were made in 1-cm silica cells with a Unicam SP500 spectrophotometer. In order to avoid errors caused by light scattering within absorption cells, all solutions must be absolutely free from dust.

Reagents—

All standard metal solutions are 0·002 *M*, *i.e.*, they contain 2 μM of metal ion per ml.

Standard magnesium solution—Dry analytical-reagent grade magnesium sulphate, $MgSO_4.7H_2O$, at 260° C overnight. Dissolve 0·240 g of the anhydrous magnesium sulphate so prepared in distilled water, and dilute to 1 litre.

Standard calcium solution—Dissolve 0·200 g of analytical-reagent grade calcium carbonate in dilute hydrochloric acid, and dilute to 1 litre.

Standard strontium solution—Dissolve 0·295 g of analytical-reagent grade strontium carbonate in dilute hydrochloric acid, and dilute to 1 litre. Alternatively, dissolve 0·533 g of analytical-reagent grade strontium chloride, $SrCl_2.6H_2O$, in distilled water, and dilute to 1 litre.

Standard barium solution—Dissolve 0·489 g of analytical-reagent grade barium chloride, $BaCl_2.2H_2O$, in distilled water, and dilute to 1 litre.

Standard zinc solution—Dissolve 0·131 g of zinc metal in dilute hydrochloric acid, and dilute to 1 litre.

Standard cadmium solution—Dissolve 0·225 g of cadmium metal in a small amount of concentrated hydrochloric acid, and dilute to 1 litre.

EDTA solution, 0·005 M—Dissolve 1·86 g of disodium ethylenediaminetetra-acetate dihydrate in 1 litre of distilled water.

Buffer solution, pH 10—Add 6 g of ammonium chloride to 57 ml of ammonia solution, sp.gr. 0·880, and dilute to 500 ml.

PREPARATION OF A CALIBRATION GRAPH—

Add solutions containing 1·0, 2·0, 3·0, 4·0 and 5·0 μM of metal ion to 1·0 ml of EDTA solution and 1·0 ml of buffer solution. Dilute each solution to 5·0 ml with distilled water, and measure the optical densities at 225 mμ against a blank solution consisting of 1·0 ml of buffer solution diluted to 5·0 ml with distilled water. At the same wavelength, measure the optical density of a solution containing 1·0 ml of EDTA solution, 1·0 ml of buffer solution and 3·0 ml of distilled water. Calculate the difference between the optical density in presence of the metal ion and that in its absence. Plot this difference against the concentration of metal ion.

PROCEDURE—

Add 1·0 ml of a solution containing between 1 and 5 μM of metal ion to 1·0 ml of EDTA solution and 1·0 ml of buffer solution. Dilute to 5·0 ml with distilled water, and measure the optical density at 225 mμ. Calculate the optical-density difference in the way described for the calibration graph. Read the amount of metal ion corresponding to this difference from the calibration graph.

TABLE I

ANALYSIS OF PURE SOLUTIONS OF METAL IONS

Magnesium—		Barium—		Cadmium—		Calcium—		Zinc—		Strontium—	
present, μM	found, μM	present, μM	found, μM	present, μM	found, μM	present, μM	found, μM	present, μM	found, μM	present, μM	found, μM
0·86	0·89	0·80	0·78	1·10	1·08	1·53	1·57	0·73	0·76	1·02	1·04
1·00	1·04	1·00	0·93	1·73	1·64	1·75	1·87	0·87	0·88	1·44	1·43
1·50	1·53	1·36	1·33	1·76	1·71	2·50	2·46	1·22	1·22	1·55	1·53
1·71	1·64	1·50	1·45	2·42	2·43	2·50	2·46	1·51	1·49	2·05	2·02
1·71	1·64	1·60	1·50	2·57	2·46	2·93	2·98	1·74	1·71	2·32	2·27
2·00	2·08	2·00	2·07	2·74	2·69	3·00	3·05	2·24	2·22	3·02	2·96
2·09	2·08	2·40	2·29	3·08	3·05	3·33	3·30	2·62	2·54	3·07	3·06
2·50	2·56	2·80	2·79	3·43	3·27	3·34	3·44	2·98	2·95	3·69	3·65
2·56	2·53	3·20	3·11	3·74	3·66	3·75	3·79	3·14	3·03	3·77	3·60
2·56	2·53	3·26	3·31	4·10	4·08	4·50	4·63	3·61	3·58		
3·00	3·07	3·40	3·45	4·29	4·14						
3·08	3·08	3·40	3·32								
3·08	3·08	4·00	4·07								
3·50	3·66	4·00	3·94								
4·00	4·16										
4·18	4·14										

Mean recovery of magnesium = 101 per cent. (standard deviation ± 3 per cent.)
Mean recovery of barium = 99 per cent. (standard deviation ± 3 per cent.)
Mean recovery of cadmium = 98 per cent. (standard deviation ± 2 per cent.)
Mean recovery of calcium = 102 per cent. (standard deviation ± 2 per cent.)
Mean recovery of zinc = 99 per cent. (standard deviation ± 2 per cent.)
Mean recovery of strontium = 99 per cent. (standard deviation ± 2 per cent.)

Results

Magnesium, calcium, strontium, barium, zinc and cadmium gave similar calibration graphs. There were slight differences in the gradients of these graphs, and this is attributed to slight variations in the absorption of the various metal chelates. The gradients of the calibration graphs for these metals were 0·202, 0·203, 0·203, 0·193, 0·205 and 0·208 optical-density units per μM, respectively.

The results of a number of determinations on pure solutions are shown in Table I, each result being the mean of duplicate determinations.

Discussion of the Method

Interfering anions—

The presence of phosphate in concentrations forty times greater than that of the metal ion did not cause any significant difference in the determination of calcium.

It is important to note that the proposed method fails in the presence of anions that absorb strongly at 225 mμ, *e.g.*, nitrate and acetate.[2]

Scope and validity of results—

The method is convenient for the rapid analysis of many samples when high accuracy is not required. An accuracy to within ± 3 per cent. can be expected in the analysis of samples containing between 1 and 5 μM of metal ion. A major disadvantage of the method is that it is not specific for a particular metal ion. In addition to the metal ion being determined, solutions for analysis may contain only the alkali metal ions (excluding lithium) and ammonium ion.

For the titration of pure solutions of magnesium, cadmium and zinc with EDTA solution, Sweetser and Bricker[1] report maximum errors of 0·91 per cent. for the determination of magnesium over the range 3·4 to 85 μM (approximately), 0·64 per cent. for the determination of cadmium over the range 9·7 to 97 μM (approximately) and 0·35 per cent. for the determination of zinc over the range 9·5 to 95 μM (approximately). The proposed method permits analyses to be carried out on smaller amounts of metal ion, but the percentage errors are somewhat greater.

One of us (E.P.) is indebted to the C.S.I.R.O. for the award of a studentship.

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The Determination of Barium and Sulphate with an E.E.L. Flame Photometer

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It is shown that suspensions of barium sulphate at concentrations of a few hundred parts per million of barium give quantitative readings with an E.E.L. flame photometer. The use of this phenomenon in the determination of sulphate ions is described, with particular reference to detergent powders.

The main purpose of this work was to devise a simple and rapid method for the determination of sulphate in detergent powders. The usual gravimetric procedure necessitates the removal of silicate, a normal constituent of such powders, and is therefore time-consuming; the volumetric method of Archer[1] and that described by Vogel[2] cannot be used in the presence of phosphate. Flame photometry seemed to be the obvious solution.

It was hoped originally that it would be possible to take an acidified solution of the detergent powder in aqueous ethanol (to prevent precipitation of barium alkylarylsulphonate),

add a measured excess of barium chloride and then determine the excess of barium by direct flame photometry, but preliminary experiments showed that a suspension of barium sulphate gave quantitative readings for barium. Attempts were then made to remove the precipitate by centrifugation and to determine barium in the clear supernatant liquid. The main difficulties that arose here were that chloride, nitrate and phosphate caused anionic interference (compare Collins and Polkinhorne[3]) even when present in low concentrations, and sodium at concentrations above 200 p.p.m. caused an increase in the scale reading, as the filter could not deal completely with such intense sodium light, although it had nominally zero transmission at 589 mμ. It was obvious that the solution would have to be virtually free from all other anions and cations, and the problem was to achieve this without unduly lengthening the procedure. This was accomplished by suspending the precipitated barium sulphate, after centrifugation, in a 1 per cent. starch solution (found to be superior to more orthodox suspending agents) and analysing this suspension directly with the instrument.

With regard to the flame photometry, the principal difficulty was the choice of filter. The filter was required to have a transmission maximum at about 493 or 548 mμ (barium lines) and to suppress the sodium lines at 589 mμ. After much correspondence with Messrs. Kodak Ltd., Wratten filters Nos. 65A and 77 (mercury monochromat) were selected as possibly having suitable properties. During experiments on determining the excess of barium, the No. 77 filter proved to be useless on account of its transmission at 589 mμ. This transmission is less than 0·2 per cent., but nevertheless it was too high, owing partly to the high sodium to barium ratio and possibly to the incomplete excitation of barium in the relatively cool flame of the E.E.L. instrument (Evans Electroselenium Ltd.). These experiments were therefore carried out with the No. 65A filter, which was retained in the later experiments with the precipitate. Unfortunately, this filter is now obsolete, but the No. 77 filter can be used in the proposed procedure, as the suspension of barium sulphate is virtually free from sodium ions. The latter filter permits the measurement of somewhat lower concentrations, on account of its higher transmission.

In all the experiments, the flame used was the standard coal gas - air flame recommended by the makers of the instrument. The use of a hotter flame, *e.g.*, coal gas - oxygen, was considered in order to achieve better excitation, but the agent from whom the instrument was purchased advised against such a procedure in view of the possibility of damaging either the burner or the photocell. This danger, however, may well be imaginary.

The method described is applicable to detergent powders of normal composition. There is no reason why, with suitable modifications, it should not be used for many other types of product.

Method

Apparatus—

An E.E.L. flame photometer, model A, and Wratten No. 65A or 77 (mercury monochromat) filters were used.

Reagents—

Hydrochloric acid, concentrated.
Diethyl ether.
Barium chloride solution, 1 per cent.
Starch solution, 1 per cent.
Standard sulphate solution—Prepare a 1·021 per cent. w/v solution of sulphuric acid or a 1·479 per cent. w/v solution of sodium sulphate. Both solutions contain 1·000 per cent. of sulphate, as SO_4^{2-}.

Procedure—

Accurately weigh about 2 g of detergent powder, and dissolve it in 100 ml of water in a 250-ml separating funnel. Add 25 ml of concentrated hydrochloric acid, and extract with four 100-ml portions of diethyl ether. Discard the ether layer, which contains the surface-active ingredient in its acid form and any fatty amides present as additives. Transfer the aqueous layer quantitatively to a 250-ml calibrated flask, dilute to the mark, and mix thoroughly.

By pipette, place a 5-ml portion in a 10-ml centrifuge tube, add 5 ml of 1 per cent. barium chloride solution, mix, and spin in a centrifuge for 3 minutes at 4000 r.p.m. and 6 inches radius. Reject the supernatant liquid. Add 10 ml of water, suspend the precipitate

by stirring thoroughly with a glass rod, and spin in a centrifuge as before, again rejecting the supernatant liquid. Add 1 per cent. starch solution exactly to the 10-ml calibration mark, and suspend the precipitate thoroughly. Use an E.E.L. flame photometer with a Wratten No. 65A or mercury monochromat No. 77 filter to analyse the suspension. Set the instrument to zero against 1 per cent. starch solution.

Prepare a standard suspension by placing 1 ml of standard sulphate solution, by pipette, in a 10-ml centrifuge tube, adding 4 ml of water and 5 ml of 1 per cent. barium chloride solution and then proceeding exactly as described previously. The final suspension contains 1000 p.p.m. of sulphate, as SO_4^{2-}, and is used to set the instrument to full-scale deflection before the test suspension is analysed. A full-scale deflection (100 scale units) is then equivalent to 1000 p.p.m. of sulphate, as SO_4^{2-}.

Calculation

The concentration of sulphate in the final suspension is found by multiplying the scale reading by 10.

The percentage concentration of sodium sulphate in the original sample is therefore given by the expression—

$$\frac{\text{Scale reading} \times 20 \times 100 \times 142}{\text{Weight of sample, g} \times 10^4 \times 0{\cdot}4 \times 96} = \frac{\text{Scale reading} \times 0{\cdot}7396}{\text{Weight of sample, g}}$$

Results

Table I shows the results for sodium sulphate solutions of known concentration.

Table I

Flame-photometric analysis of sodium sulphate solutions

Sulphate present, as SO_4^{2-}, p.p.m.	Scale reading with Wratten No. 65A filter	Scale reading with Wratten No. 77 filter
0	0	0
100	10	9
200	19	19
300	29	—
400	39	—
500	50	50
600	59	—
700	70	69
800	81	—
900	90	—
1000	100	100

Table II shows values for the recovery of added sulphate from sulphate-free alkylarylsulphonate and also a comparison of results found for detergent powders by the proposed method and by the usual gravimetric procedure. The three detergent powders contained only sodium alkylarylsulphonate as active ingredient.

Table II

Recovery of added sodium sulphate and analysis of detergents

Sample No.	Sodium alkylarylsulphonate present, %	Silicate present, as $Na_2Si_2O_5$, %	Phosphate present, as P_2O_5, %	Sodium sulphate added, p.p.m.	Sodium sulphate found by flame photometry— p.p.m.	%	Sodium sulphate found gravimetrically, %
Sodium alkylarylsulphonate—							
1	100	—	—	300	300	—	—
2	100	—	—	400	400	—	—
3	100	—	—	500	490	—	—
Detergent powders—							
1	33·0	4·75	18·0	—	—	13·8	13·6
2	29·2	Nil	17·1	—	—	33·3	33·3
3	20·7	3·80	17·4	—	—	20·6	20·6

The proposed procedure could obviously be applied to soap powders, although sodium sulphate is not normally an ingredient of such products. It is probably applicable to detergent powders containing active ingredients other than alkylarylsulphonate—for example, it is known from experience in other types of analysis that the extraction procedure described will remove substantially all fatty alcohol sulphate from the aqueous layer—but no such powder was examined, as they are at present comparatively rare.

In one of the preliminary experiments, a 50-ml portion of a barium chloride solution containing 2000 p.p.m. of barium, as Ba^{2+}, was placed, by pipette, in each of nine 100-ml calibrated flasks. By pipette, 0, 5, 10, 15, 20, 25, 30, 35 and 40-ml portions of sulphuric acid containing 2000 p.p.m. of sulphate, as SO_4^{2-}, were added, respectively, to the flasks. Each solution was diluted to the mark, mixed and spun in a centrifuge. The supernatant liquids (each having initially contained 1000 p.p.m. of barium) were analysed; that to which no sulphate had been added was used to set the instrument to full-scale deflection. The results were as follows (note that 1000 p.p.m. of Ba^{2+} are equivalent to 699 p.p.m. of SO_4^{2-})—

Sulphate present, p.p.m.	0	100	200	300	400	500	600	700	800
Scale reading	100	86	73	59	45	30	15	0	0
Calculated excess of barium, p.p.m.	1000	857	714	571	428	285	142	0	0

Application of the method

Determination of barium—

In view of the already mentioned sensitivity of the barium flame to anionic interference, it is to be expected that, when solutions or suspensions containing a constant concentration of barium ions are analysed by flame photometry, the scale reading will vary with changes in the anion, even in the complete absence of other salts. This was confirmed by experiments on aqueous solutions of barium chloride and suspensions of barium sulphate in 1 per cent. starch solution. These were analysed in turn, both the No. 65A and 77 filters being used.

With barium chloride solutions, the minimum concentrations of barium that would give full-scale deflection were 700 p.p.m. when the No. 65A filter was used and 500 p.p.m. when the No. 77 filter was used; with barium sulphate suspensions, the corresponding minimum concentrations were 1100 and 800 p.p.m., respectively. The results are shown in Table III.

Table III

Determination of barium

Barium chloride solution			Barium sulphate suspension		
Barium present, as Ba^{2+}, p.p.m.	Scale reading with No. 65A filter	Scale reading with No. 77 filter	Barium present, as Ba^{2+}, p.p.m.	Scale reading with No. 65A filter	Scale reading with No. 77 filter
0	0	0	0	0	0
100	10	10	100	9	9
250	25	25	200	18	20
500	50	50	400	36	40
750	75	75	500	45	50
1000	100	100	700	62	68
—	—	—	1000	90	100
—	—	—	1100	100	—

For each series of experiments except that with barium sulphate and the No. 65A filter, the solution containing 1000 p.p.m. of barium was used to set the flame photometer to full-scale deflection. For the barium sulphate and the No. 65A filter, this was done with the suspension containing 1100 p.p.m. of barium.

In all instances, the minimum concentration for full-scale deflection is many times greater than that for sodium, potassium, lithium, calcium and so on, which give full-scale deflection at concentrations between 5 and 20 p.p.m. When more sensitive instruments are used, similar results can be achieved for barium, and the insensitivity of the E.E.L. instrument is, as previously suggested, probably due to incomplete excitation in the relatively cool flame.

We thank Colgate - Palmolive Ltd. for permission to publish this paper.

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Notes

DIRECT TITRATION OF HYDROXYL GROUPS WITH LITHIUM ALUMINIUM DI-*n*-BUTYL AMIDE

LITHIUM aluminium di-*n*-butyl amide was shown by Higuchi, Concha and Kuramato[1] to be sufficiently strong a base to titrate hydroxyl hydrogen as an acid in ether solvents. As this reagent lacks the reducing properties of lithium aluminium hydride and does not generally react with amino hydrogen, it was found to be useful for the analysis of compounds in the ethanolamine series.

In the original method, the reagent was used in the form of a 1·0 *N* solution in tetrahydrofuran. An excess of this solution was added, by pipette, and titrated back with a standard butyl alcohol - xylene solution.

When some ethanolamines were titrated in this manner, the end-points were unstable. It was suggested by Higuchi (in a private communication) that this might be caused by temporary displacement of amino hydrogen by the excess of reagent, the resulting base then reacting more slowly during titration with the butyl alcohol - xylene solution. Attempts to avoid an excess of reagent by using direct titration showed that the reagent blocked the tip of the burette to such an extent that it could not be dispensed in this manner. The nature of the solvent made the reagent unsuitable for use in burettes having greased stopcocks or rubber connections. A study was therefore made to adapt the reagent to direct titration. It was found that, when dimethoxyethane was used as solvent and the concentration of the solution was reduced to 0·25 *N* or less, the reagent could be dispensed from a burette having a Teflon stopcock, which has recently become commercially available. When direct titration was used, end-points were sharp and definite.

METHOD

APPARATUS—

The apparatus used is shown in Fig. 1. A 10-ml automatic gravity-filling burette having a Teflon stopcock (obtained from Fischer and Porter Co., Hatboro, Pa.) was used. The ground-glass stopper of the reservoir was equipped with a fitted standard taper sleeve of Teflon film (obtained

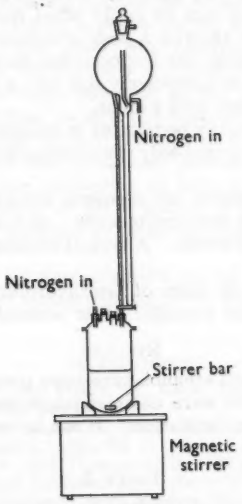

Fig. 1. Titration apparatus

from Arthur F. Smith Co., Rochester 4, New York). The titration vessel consisted of a 500-ml resin pot with cover. The four openings in the cover were fitted with rubber stoppers, one containing the nitrogen inlet tube and another bored to admit the burette tip loosely. A magnetic stirrer with a Teflon-covered stirring bar was used, and dry nitrogen was supplied to the apparatus at a pressure of 5 to 10 inches of water.

REAGENTS—

Dimethoxyethane—Ansul ether 121 (obtained from Ansul Chemical Company, Marinette, Wisconsin) was used.

Tetrahydrofuran.

Lithium aluminium hydride.

Di-n-butylamine.

Indicator solution—A 0·1 per cent. w/v solution of 4-phenylazodiphenylamine (obtained from Distillation Products Industries, Rochester 3, New York) in benzene was used.

PROCEDURE—

Distil at least 300 ml of dimethoxyethane from a slight excess of lithium aluminium hydride in an apparatus fitted with ground-glass joints and vented to atmosphere through a drying tube. Indicator may be added to show the presence of excess of lithium aluminium hydride before distillation; for safety reasons, do not distil to dryness. Place 300 ml of the freshly distilled solvent in a 500-ml flask fitted with a ground-glass joint, add 1·0 g of lithium aluminium hydride, attach a vertical condenser, and boil under reflux for 20 minutes. Add 20 ml of di-*n*-butylamine very slowly via the condenser (decrease the amount of heat if necessary), continue to boil under reflux for 10 minutes, and then allow to cool. (To allow solids to settle, it is advantageous to set the solution aside for 1 hour in an atmosphere of nitrogen at this stage.) Thoroughly displace air from the burette with nitrogen, and filter the solution through glass-wool into the reservoir. Close the reservoir, and maintain it under slight pressure of nitrogen.

Place 200 ml of tetrahydrofuran (distilled in the manner described) in the resin pot under an atmosphere of nitrogen. Add a few drops of indicator solution, start the magnetic stirrer, and titrate to the end-point; the colour change is from yellow to red. Weigh the sample into the resin pot from a weighing pipette or other convenient device, allow time for mixing or dissolution, and again titrate to the end-point. Standardise the solution by titrating a sample of butyl alcohol.

DISCUSSION OF THE METHOD

Some difficulty has been caused by blockage of the burette tip. The burette is therefore mounted in such a way that the tip can be easily lifted from the resin pot for clearing. It is usually sufficient to wipe the burette tip with a piece of cotton; after prolonged standing, sediment is removed with a fine wire. Blockage has not occurred during a titration. The burette as obtained from the manufacturer had an excessively fine tip; a portion of this was broken off, and the end was ground to a suitable taper with a stone.

A dozen or more samples can usually be titrated in succession, the same tetrahydrofuran being used. In general, fresh solvent is required only when end-points are unsatisfactory or precipitation is excessive.

When the burette is left overnight, the meniscus should be lowered below the graduation marks, as some deposition occurs on the burette walls. If it is adequately protected by nitrogen, the solution can be used for at least 2 weeks. A typical solution decreased from 0·2360 to 0·2040 *N* in 6 days.

Dimethoxyethane can be used in place of tetrahydrofuran in the resin pot if desired, and several variations of the reagent are possible; other amines[1,2] have been used.

RESULTS

The results of analyses of typical ethanolamine-type compounds by the proposed procedure are shown in Table I. The samples were commercial materials, with the exception of monoethanolamine, which was purified by distillation. It can be seen that the precision of the method is somewhat better than 1 per cent.

TABLE I

DETERMINATION OF ACTIVE HYDROGEN IN ETHANOLAMINE-TYPE COMPOUNDS

Sample	Amount of active hydrogen found, equivalents per mole
Monoethanolamine	0·988, 0·985
Diethanolamine	1·910, 1·915, 1·910
N-ethylethanolamine	1·048, 1·058

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Arnold, Hoffman & Co. Inc.
(Subsidiary of Imperial Chemical Industries Ltd.)
55, Canal Street, Providence 1
Rhode Island, U.S.A.

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COLOUR TEST FOR THE DETECTION OF HYPONITRITES

During a study of the oxidation and reduction of the lower and higher oxidation states of nitrogen, a test for detecting hyponitrite was required.[1] As far as we are aware, the only test for the detection of small amounts of hyponitrite is that described by Corbet[2] and by Gopala Rao and Sandara Rao.[3] According to Corbet, hyponitrites in the presence of potassium periodate and resorcinol form an orange colour in acid and a green colour in alkaline solutions. These reactions should make it possible to detect a concentration of 1 p.p.m. of hyponitrite. Gopala Rao and Sandara Rao recommend that the test be carried out in a solution buffered at pH 2 to 5. As hyponitrites are unstable[4] in acid solutions and as the nature of the colour reaction is uncertain,[2,3] we have determined the best conditions for the reaction and its degree of specificity.

The procedure is described below. Twenty millilitres of Sörensen's citrate buffer mixture or 0·01 *N* sodium hydroxide were mixed with 1 ml of freshly prepared 0·1 *M* resorcinol, 1 ml of a saturated solution of potassium periodate and 1 ml of 0·05 *M* sodium hyponitrite or sodium nitrite. Thirty minutes after its preparation, the optical density of each solution was measured over the wavelength range of 400 to 700 mμ with a Zeiss Universal spectrophotometer. Sodium hyponitrite was prepared and purified by the procedure described by Addison, Gamlen and Thompson.[5] Analytical-reagent grade resorcinol was recrystallised from glass-distilled water and then sublimed twice *in vacuo*.[6] (Merck's tests[7] were used to verify its purity.) All other chemicals were of analytical-reagent grade.

We found that the colour produced by the reaction of hyponitrites with resorcinol and potassium periodate in acid solution is not specific for hyponitrites, but is for nitrites. In acid solution containing resorcinol and nitrite, a yellow colour is formed and changes to red in the presence of potassium periodate. The absorption spectrum of the coloured solution has a characteristic maximum at 513 mμ, the position of which does not change with the pH of the solution. The optical density at 513 mμ decreases as the pH increases. Other nitrogen compounds that are oxidised by periodate to nitrite[8,9] therefore produce exactly the same colour reaction with resorcinol and periodate in acid solution as does hyponitrite.

In alkaline solutions, resorcinol and potassium periodate form a coloured solution in the absence of nitrogen compounds. The absorption of light by such solutions varies considerably with the pH, the absorption spectrum at pH 8 having a maximum at 498 mμ and at pH 12 maxima at 456 and 623 mμ. The positions of these maxima are not affected by the presence of nitrites or hyponitrites.

As the only published reaction for hyponitrites[2,3] proved to be a reaction for nitrites, it is not possible to identify small amounts of hyponitrite formed in the presence of nitrite, oxyhyponitrite[5] or hydroxylamine.

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Book Reviews

STABILITY CONSTANTS OF METAL-ION COMPLEXES, WITH SOLUBILITY PRODUCTS OF INORGANIC SUBSTANCES. Compiled by JANNIK BJERRUM, GEROLD SCHWARZENBACH and LARS GUNNAR SILLEN under the Auspices of The International Union of Pure and Applied Chemistry. PART II: INORGANIC LIGANDS. Compiled from the literature up to the middle of 1957, with collaboration by CLARA BERECKI-BIEDERMANN, LORENS MALTESEN, SVEND ERIK RASMUSSEN and FRANCIS J. C. ROSSOTTI. Pp. xvi + 131. London: The Chemical Society, 1958. Price 40s. (Special Publication No. 7).

Part I of these Tables (*Analyst*, 1958, **83**, 381) summarised the data for 464 organic ligands. Although the present volume deals only with the reactions of 56 inorganic ligands ranging from F^- and OH^- to $Hg(SCN)_4^{2-}$ and $(P_4O_{12})^{4-}$, many inorganic ligand - metal ion systems have been studied repeatedly and the wealth of data which proves an embarrassment to the research worker must have proved an almost insuperable obstacle to the compilers of this fine work. Thus under the heading Cl^- and Ag^+ there are no less than 48 numerical values derived from 44 different publications!

Since equilibrium in inorganic systems often depends upon solubilities or acid - base properties, the solubility products of sparingly soluble substances and ligand - proton association constants have been included, as well as equilibrium data for such processes as $HgI_2(s) + I^- \rightleftharpoons HgI_3^-$, which may serve as a basis for further calculations. Thermodynamic data for ΔH and ΔS are quoted whenever available. Hydrolysis constants for metal ions are described in terms of the hydroxyl ion as one of the ligands. The pronounced tendency to form polynuclear complexes, especially when hydroxide or silver ions are concerned, must have added greatly to the authors' difficulties, but the excellent introductory pages (ix to xvi) entitled "How to use the Tables" are so clearly written that the new reader will soon learn to find his way about and to handle with confidence any one of the numerous types of equilibrium he may encounter.

For each metal - ligand combination is given the temperature of measurement, the medium (under 14 headings), the method of measurement (under 48 different headings!), the particular reaction under discussion and the full literature references. The two volumes of this series form an epoch-making contribution to chemical literature, and chemists of all kinds must pay high tribute to the industry, the expertise—and the courage and determination—of those who have thus made the world's literature available on our bookshelves. Can anyone afford *not* to possess this new volume?

H. IRVING

MECHANISM OF INORGANIC REACTIONS: A STUDY OF METAL COMPLEXES IN SOLUTION. By FRED BASOLO and RALPH G. PEARSON. Pp. xiv + 426. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1958. Price $11.75; 94s.

Most analytical procedures pay considerable attention to the time factor. In gravimetric analysis time is allowed for completing the precipitation and for "ageing" effects. In absorptiometry the effect of concentration on the rate of colour development and the possibilities of "fading" must be included in the recommended procedure. Catalysts are used to speed some reactions and inhibitors to retard others. Deliberate use is sometimes made analytically of a marked difference in the speeds of two reactions. Yet despite the importance of this topic, the study of the kinetics of inorganic reactions has lagged far behind that of organic reactions and only in the last two decades has work in this field become widespread. Although a little of this work has been carried out on problems of immediate analytical interest (*e.g.*, the hydrolysis of thioacetamide as a source of S^{2-}, or the rates of electron transfer between ions of the same element in different valency states—which are of such significance in the applications of radionuclides) it must be admitted that the greatest advances have been achieved from academic studies carried out for the most part with co-ordination compounds of various types.

Basolo and Pearson's review of the present position is timely and satisfying. Although it is not written for the analyst (and indeed nowhere suggests that the topic could be of possible interest to him) it can be read with profit by anyone prepared to face and assimilate a rather bewildering mass of new facts and a great deal of theory, which is presented clearly but perhaps over-concisely for anyone completely unfamiliar with the current ideas on, *e.g.*, ligand field theory. With luck it may stimulate someone to write a book on reaction kinetics aimed at the analytical chemist. At the very least it should encourage some workers to apply the techniques and ideas it so ably describes to what is still largely a virgin field.

H. IRVING

COLORIMETRIC DETERMINATION OF NONMETALS. Edited by DAVID F. BOLTZ. Pp. xii + 372. New York and London: Interscience Publishers Inc. 1958. Price $8.50; 65s.

One wonders if the method adopted in the preparation of this book is symptomatic of general practice in the near future. Notwithstanding the circumscribed subject, this is the joint work of fourteen authors. There can be no doubt of the excellence of this approach for presenting a rapidly expanding subject, since it minimises the difficulty, so often encountered in scientific work, of books being out of date even before they are published.

The scope can be indicated by listing the chapter headings; the number of pages devoted to each element is shown in brackets. After a preliminary section devoted to general principles the real business begins with a chapter on phosphorus (18) and then, successively, silicon (28), nitrogen-ammonia, nitrite and nitrate (86), chlorine (17), bromine (16), iodine (34), fluorine (30), sulphur (48), tellurium and selenium (30) and boron (15). It should be remarked that in many cases it is the anion that is determined and not always the element itself; thus phosphorus is determined as phosphate and silicon as silicate, but the chapter on chlorine includes assessment of hypochlorite, chloride, chlorate and perchlorate ions, as well as the free element.

The editor has successfully standardised the method of presentation of these excellent monographs, which begin by a full general discussion followed by methods of separation from diverse materials and conclude with detailed working descriptions of procedures applicable to various circumstances. Copious references to the original literature are given throughout.

In the preface it is stated that ". . . the authors have presented methods based upon their experience and/or judgment which they believe to be the most suitable." If, in some cases, our own favourites are not described, we should undoubtedly consider the procedures given as worthy of trial, particularly as those chosen are not necessarily the latest recorded in the literature. Because the monographs comprising this book have been written by different authors, it is hardly appropriate to pass critical comments on details, but perhaps one might venture to suggest that when a second edition is being prepared the phenoldisulphonic acid method for nitrate be supplemented by a description of the modified procedure due to R. C. Frederick (*Analyst*, 1919, **44**, 281), which is applicable in the presence of even 0·1 per cent. of chloride. Again, of the numerous procedures proposed for the colorimetric determination of phosphates, the reviewer is inclined to favour an organic amino reducer, such as metol, rather than hydrazine sulphate because he believes the interference due to silicate is somewhat less pronounced.

In conclusion, it should be observed that this book is well produced and will, without doubt prove to be of real value to analysts in many fields of activity. NOEL L. ALLPORT

SPOT TESTS IN INORGANIC ANALYSIS. By F. FEIGL, Eng., D.Sc. Translated by R. E. OESPER, Ph.D. Fifth Edition. Pp. xiv + 600. Amsterdam: Elsevier Publishing Co.; London: Cleaver-Hume Press Ltd.; New York and Toronto: D. Van Nostrand Co. Inc. 1958. Price 65s.

This was formerly Volume I (Inorganic Applications) of Feigl's "Spot Tests"; it now appears as a separate work, as the Organic Applications did in 1956. The general arrangement of the book remains the same as in previous editions and needs no description.

All the sections have been expanded and brought up to date. The chapter on techniques now includes an account of the ring oven. There are new tests for almost all of the 42 cations listed. The number of tests in this section has been increased to almost 300, although much of this new material is in "other tests," where procedures are not so fully described. Five new anions are included (cyanate, hypohalite, perchlorate, dithionite and sulphamate), and there are new tests for the 30 others listed. There are several additions to the tests for free elements, both metal and non-metal, and the number of specific applications of spot tests has been increased to nearly one-hundred. The valuable tabular summary of tests with their identification limits, in effect a second index, has been brought up to date.

If there be any inorganic analyst who is not familiar with this book, he may gauge something of its merits from the fact that there are over 1100 references to original work. That this is an increase of over 200 since the last edition 4 years ago is a measure of the continued expansion of the subject and of the diligence of the author.

The new material, in conjunction with the clarity of the text—a tribute to both author and translator—and the high quality of production, enhances the already established position of "Spot Tests." It is valued not only by those who use spot tests consciously as such, but by a great company of analysts who use it as a reference book on a multitude of qualitative, and on some quantitative, problems. DAVID W. WILSON

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POLAROGRAPHY IN MEDICINE, BIOCHEMISTRY AND PHARMACY. By M. BREZINA and P. ZUMAN. Pp. xviii + 862. New York and London: Interscience Publishers Inc. 1958. Price $19.50; 146s.

PLASTICS ABSTRACTS. Volume I. Number I. Abstracts 1–72. 13th January, 1959. Welwyn, Herts.: Plastics Investigations (23 Canonsfield Road). Subscription rates to Plastics Abstracts are £25 annually, including postage, or single copies may be obtained price 10s.

Alternative rates are £35 by air-mail. Copies printed on one side only can be supplied for £30 annually, or £45 by air-mail. Six months' subscriptions are at half the annual rate, single copies by air-mail 14s.

REPRINTS OF "THE ANALYSIS OF SYNTHETIC DETERGENTS" BY W. B. SMITH

REPRINTS of the Review Paper, "The Analysis of Synthetic Detergents," by W. B. Smith, published in this issue of *The Analyst* (pp. 77 to 89), will be available shortly from the Assistant Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 2s. 6d. per copy, post free. A remittance for the correct amount, made out to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not obtainable through Trade Agents.

Erratum

JULY (1958) ISSUE, p. 424, 21st line and 2nd line of legend to Fig. 2. *For* "ethylbis(2-chloroethyl)amine" *read* "methylbis(2-chloroethyl)amine."

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1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, **1933**, **52**, 189T.
2. Mitchell, C. A., *Editor*, "Allen's Commercial Organic Analysis," Fifth Edition, J. & A. Churchill Ltd., London, **1932**, Volume **9**, p. **149**.

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